Synaptic Plasticity in Local Cortical Network In Vivo and Its Modulation by the Level of Neuronal Activity

Neocortical neurons maintain high firing rates across all behavioral states of vigilance but the discharge patterns vary during different types of brain oscillations, which are assumed to play an important role in information processing and memory consolidation. In the present study, we report that trains of stimuli applied to local neocortical networks of cats, at frequencies that mimic endogenous brain rhythms, produced depression or potentiation of postsynaptic potentials, which lasted for several minutes. This form of synaptic plasticity was not mediated through NMDA receptors since it persisted after blockade of these receptors, but was strongly modulated by the level of background neuronal activity. Using different preparations in vivo, we found that increased background neuronal activity decreased the probability of plastic changes but enhanced the probability of potentiation over depression. Conversely, when the level of background neuronal activity was low, plasticity was observed in all neurons, but mainly depression was induced. Our results demonstrate that high levels of neuronal activity in the cortical network promote potentiation and insure the stability of synaptic connections.

Keywords: Intracellular, neocortex, oscillations, plasticity, slow-wave sleep

Introduction

One of the intriguing features of neocortical activities is that these neurons maintain a relatively high level of spontaneous discharge across all states of vigilance, including the disconnected state of slow-wave sleep (SWS) (Steriade et al., 2001). This sleep state has long been associated with global inhibition of the cerebral cortex and subcortical structures that underlies the annihilation of consciousness. However, far from being a completely quiescent state, SWS appears to be a state during which active mental processes take place (Hobson and Pace-Schott, 2002; Steriade and Timofeev, 2003; Huber et al., 2004). Studies using extra- and intracellular recordings of neocortical neurons in naturally awake and sleeping cats have demonstrated that mean firing rate shows little change from one behavioral state to another, whereas the pattern of discharge during both brain-activated states, i.e. waking and rapid-eye-movement (REM) sleep, is clearly distinct from that of SWS (Steriade, 1978; Destexhe et al., 1999; Steriade et al., 2001; Timofeev et al., 2001).

Neocortical neurons are engaged in corticothalamocortical loops, which generate different rhythmic activities according to the state of vigilance. These oscillations drive neocortical neurons to fire in a more or less synchronous manner, specific to each behavioral state (Silva et al., 1991; Singer, 1993, 1999; Steriade, 1999; Buzsáki and Draguhn, 2004). Typically, neocortical activities are dominated by fast oscillations in the β/γ range (20–80 Hz) during activated states, whereas spindles (7–14 Hz), delta (1–4 Hz), fast (20–80 Hz) and ultra-fast oscillations (ripples, 80–200 Hz) are grouped by the slow oscillation (0.5–1 Hz) during SWS (Steriade et al., 1993a,b; Steriade and Amzica, 1998; Steriade, 2000; Grenier et al., 2001). The grouping of these rhythms by the slow cortical oscillation, reported in these animal experiments, was also observed in recordings during human sleep (Mölle et al., 2002). The slow rhythm is of cortical origin, since it survives thalamectomy (Steriade et al., 1993b) and is present in the isolated cortex in vivo (Timofeev et al., 2000) and in vitro (Sanchez-Vives and McCormick, 2000). This oscillation consists of an alternation of hyperpolarizing phases (also called ‘down’ states), during which all cortical neurons are silent, including identified short-axonated aspiny interneurons (Contreras and Steriade, 1995), and depolarizing phases (also called ‘up’ states), which repeat every 1–2 s.

Studies in our laboratory have shown that brain oscillations are not simply epiphenomena, but play an important role in information processing, sustained changes in neuronal excitability and synaptic transmission (Steriade, 2000; Steriade and Timofeev, 2003). For instance, 10 Hz stimulation applied to the thalamus or the local neocortical networks produces augmenting responses, a form of short-term synaptic plasticity that results in progressively increasing cortical responses (Morison and Dempsey, 1943; Steriade et al., 1998; Timofeev et al., 2002). Moreover, the enhancement of responses that occurs during augmenting potentials or spontaneous spindles may persist for several minutes. However, in those studies the effect of rhythmic stimulations was only investigated for the frequency of 10 Hz. More recently we found that rhythmic pulse-trains at frequencies from 10 to 500 Hz applied to the contralateral cortex induced a sustained (several minutes) and NMDA receptor-dependent enhancement of postsynaptic potentials (PSPs) recorded from neurons in the homotopic cortex (Cissé et al., 2004).

The different types of synaptic plasticity in neocortex have been investigated intensively in vitro. Such studies have revealed a wide range of mechanisms that include changes at the postsynaptic level mediated through NMDA receptors (Hirsch and Crepel, 1991) or metabotropic glutamate receptors (mGluRs) (Vickery et al., 1997; Bordi and Ugolini, 1999), changes at the presynaptic level mediated by mGluRs (Bordi and Ugolini, 1999), as well as heterosynaptic plasticity mediated by mGluRs or GABA_b receptors (Bordi and Ugolini, 1999; Chu and Hablitz, 2003). In addition, the increase in intracellular Ca2+ concentration ([Ca2+]i) required for plasticity at the postsynaptic site can be achieved by interplay between PSP, back-propagating action potentials (APs) and voltage-dependent...
Ca²⁺ currents at the dendritic level (Schiller et al., 1997; Reyes, 2001; Sabatini et al., 2001; Larkum and Zhu, 2002). All these mechanisms, which most probably coexist in the cortical network, are difficult to dissociate in the in vivo preparation. Furthermore, the rich ongoing neuronal activity in the cortical network in vivo probably maintains synapses in a condition of steady-state plasticity that should affect the establishment of further plasticity (Galarreta and Hestrin, 1998). Our goal in the present study was to determine whether stimulations that mimic spontaneously occurring rhythms are able to produce plastic changes in PSP under physiological conditions and how this change can be affected by the level of background neuronal activity that characterizes the cortical network in vivo.

Materials and Methods

Animal Preparation

Experiments were conducted on 30 adult cats, under barbiturate or ketamine-xylazine anesthesia, and paralyzed with gallamine triethiodide. In order to investigate the effect of the level of neuronal activity in the cortical network on synaptic plasticity, we performed experiments in four preparations: (i) intact cortex under pentobarbital sodium (20 mg/kg, i.v.) anesthesia; (ii) intact cortex under a mixture of ketamine-xylazine (10–15 and 2–3 mg/kg i.m., respectively), and pentobarbital sodium (20 mg/kg, i.v.); (iii) intact cortex under ketamine-xylazine anesthesia (10–15 and 2–3 mg/kg i.m., respectively); and (iv) a small isolated cortical slab (12 × 6 mm; see Timofeev et al., 2000) under ketamine-xylazine anesthesia. In all preparations, the cats were ventilated artificially with the control of end-tidal CO₂ at 3.5–3.7%. The body temperature was maintained at 37°–38°C and the heart rate was 90–100 beats/min. Stability of intracellular recordings was ensured by the drainage of cisterna magna, hip suspension, bilateral pneumothorax and filling the hole made for recordings with a solution of 4% agar. At the end of experiments, the cats were given a lethal dose of pentobarbital. The experimental protocols were approved by the Committee for Animal Care and Protection of Laval University (permission no. 2002-007) and also conformed to the policy of the American Physiological Society.

Intracellular Recordings and Stimulation

Intracellular recordings from left suprasylvian association areas 5 and 7 were performed using glass micropipettes filled with 0.7 M potassium acetate (KAc). A high-impedance amplifier with active bridge were performed using glass micropipettes filled with a solution of 3 M (permission no. 2002-007) and also conformed to the policy of the dose of pentobarbital. The experimental protocols were approved by the drainage of cisterna magna, hip suspension, bilateral

Test stimuli were then applied again for 3–60 min. Because of the variability of the response amplitude, at least 30 successive responses before and 30 responses after the conditioning protocol were used for statistical analysis.

The statistical significance of the change in amplitude of responses after conditioning protocols was determined using one-way analysis of variance (ANOVA) and two-tailed t-test; when significant, the change in amplitude is expressed as a percentage of the control (before conditioning) amplitude. The statistical significance of the change in PSP amplitude was computed pulling together all responses, regardless the occurrence of the stimulation during spindles or inter-spindle lull under barbiturate anesthesia or during the depolarizing or the hyperpolarizing phase of the slow oscillation under ketamine-xylazine anesthesia. Because of the variability of the response amplitude, at least 30 successive responses before and 30 responses after the conditioning protocol were used for statistical analysis.

The proportions of neurons displaying potentiation, depression or no change in different conditions were compared with a chi-square (χ²) test. To investigate the contribution of inhibition in the establishment of plasticity, we have computed for most responses a ratio of the excitatory and inhibitory component of the response. The position of the peak was determined in the average response. The method to compute the reversal potential and changes in input resistance (Rᵢ) during the response is described elsewhere (Fuentealba et al., 2004). Briefly, the Vᵢ of the cell was set at different levels by injecting positive and negative DC currents. The bridge balance was checked and adjusted for each DC current injection. The reversal potential and Rᵢ were computed from averaged responses (n = 10–20) selected during silent periods of the cortical network, i.e. inter-spindle lull under barbiturate anesthesia and hyperpolarizing phase under ketamine-xylazine anesthesia.

Data Analysis

The responses elicited by cortical stimulation typically consisted of excitatory and inhibitory postsynaptic potentials (EPSP-I PSP sequence) (Figs 1 and 2). Since Vᵢ fluctuations affect strongly the amplitude of responses, only neurons with <5 mV changes in their mean Vᵢ were retained for analysis. Although the instantaneous Vᵢ of cortical neurons recorded in vivo fluctuates due to spontaneous activities, the mean Vᵢ was stable over time in most of the recordings. Instantaneous Vᵢ fluctuation is the major factor accounting for the high variability of the amplitude of individual elicited PSP in vivo. As illustrated in Figures 3, 5 and 6, the variability of the PSP amplitude was correlated with the amplitude of instantaneous Vᵢ fluctuations: the highest variability was observed in neurons recorded under ketamine-xylazine and the lowest variability was in neurons recorded in the isolated cortical slab.

The amplitude of individual responses was measured from the baseline Vᵢ (just before stimulation) to the peak of the excitatory component of the response. The position of the peak was determined in the average response. The method to compute the reversal potential and changes in input resistance (Rᵢ) during the response is described elsewhere (Fuentealba et al., 2004). Briefly, the Vᵢ of the cell was set at different levels by injecting positive and negative DC currents. The bridge balance was checked and adjusted for each DC current injection. The reversal potential and Rᵢ were computed from averaged responses (n = 10–20) selected during silent periods of the cortical network, i.e. inter-spindle lull under barbiturate anesthesia and hyperpolarizing phase under ketamine-xylazine anesthesia.

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the fast-rhythmic-bursting (FRB) type. Only a few fast spiking (FS) neurons were found in recordings satisfying our criteria and therefore these neurons were not included in the database. Some neurons were stained with intracellular injection of Neurobiotin and most of them were pyramidal neurons. Neurons were located in all layers of the cortex from II to VI, with predominance in layers II/III.

Characterization of Cortically Elicited Responses

We used electrical stimulation applied trough bipolar electrodes in the vicinity of the recorded neurons. The intensity of the stimulation was adjusted to approximately half of the intensity producing the maximal response. Each response was therefore due to the activation of a pool of neurons and axons and could mimic the synchronous firing of cortical neurons during endogenous oscillations. The conditioning protocol used to induce synaptic plasticity was the repetition every 2 s of trains of five stimuli at various frequencies in the range of endogenous rhythms: 10 Hz for spindles, 40 Hz for the γ oscillation, and 100 Hz for ripples. The idea was to mimic the grouping of these rhythms by the slow oscillation (0.5–1 Hz).

Electrical stimuli applied in the vicinity of the recording micropipette elicited typically a biphasic response that consisted in a sequence of depolarizing and hyperpolarizing PSP. Figure 1 illustrates a typical response in barbiturate-anesthetized animals: the early excitatory component was rapidly shunted by a powerful inhibition, as revealed by depolarizing the cell with DC current injection. This biphasic response was very similar to spontaneous events (Fig. 1B), indicating that stimulation elicited physiological-like responses. To better characterize cortically elicited responses, we recorded the neurons under different DC current injections while stimulating the cortex. We were then able to compute the reversal potential and the change in the apparent $R_{in}$ throughout the response. Under barbiturate anesthesia, the whole response was associated with strongly decreased $R_{in}$. The reversal potential of the response decreased from $-50/-60$ mV for the early depolarizing component to $-70/-80$ mV for the

Figure 1. Characterization of cortically elicited responses under barbiturate anesthesia. (A) Depth electroencephalogram (EEG) and intracellular recording of a cortical neuron. (B) Stimuli applied to the ipsilateral cortex elicited compound responses (left) similar to spontaneously occurring events (right). The cell was held at three different $V_{m}$s, by injection of DC current, to reveal the inhibitory component of the responses. (C) The left panel shows the average responses recorded under three levels of DC current. The right panel shows an average response (black) and the instantaneous change in input resistance ($R_{in}$) during the response expressed as percentage of control. The lower trace shows the time course of the reversal potential of the response. Note the reversal potential of the initial depolarizing peak of the response around $-50$ mV, whereas the long hyperpolarizing potential, associated with a strong decrease in $R_{in}$, reverses between $-70$ and $-80$ mV.
hyperpolarizing component (right panel in Fig. 1C), characteristic of a GABA<sub>A</sub>-mediated IPSP.

Similarly to responses recorded under barbiturate anesthesia, those recorded under ketamine–xylazine anesthesia consisted of a depolarizing-hyperpolarizing sequence, which looked like spontaneously occurring events (Fig. 2B). However, the whole response was not associated with a decrease in \( R_{\text{in}} \); instead, the late hyperpolarizing potential was associated with increased \( R_{\text{in}} \) (right panel in Fig. 2C). The early component of the response was associated with an increased \( R_{\text{in}} \), with a reversal potential of 

\[-50/60 \text{ mV} \]

becoming rapidly more hyperpolarized at 

\[-70/80 \text{ mV} \]

after the peak. This indicates that the early excitatory response under ketamine–xylazine anesthesia is also shunted by a powerful IPSP. The late hyperpolarizing component of the response associated with an increase in \( R_{\text{in}} \) is due to a global decrease of activity in the local cortical network previously reported as 'disfacilitation' (Contreras et al., 1996). Disfacilitation, which is also responsible for the hyperpolarizing phase of the slow oscillation, referred to periods during which cortical neurons in the local network, including inhibitory interneurons, are silent. Consequently, the number of synaptic inputs received by the cortical neurons is dramatically reduced and the \( V_m \) is set mainly by intrinsic currents, such as leak K<sup>+</sup> currents. Because of the increase in \( R_{\text{in}} \) during disfacilitation, the hyperpolarizing potential cannot be reversed by hyperpolarizing the cell, but instead its amplitude increases when injecting hyperpolarizing DC current into the cell (see Fig. 2B,C, lower traces).

Cortically Elicited Synaptic Plasticity under Barbiturate Anesthesia

We have previously reported that trains of stimuli from 10 to 500 Hz applied repetitively to the contralateral cortex produce a marked potentiation of the response elicited by that stimulus, involving NMDA receptors (Cissé et al., 2004). In the present study, we first wanted to determine whether such
plasticity can be induced by stimulation of the local (ipsilateral) cortical network and recorded 26 neurons in the association cortical areas 5 and 7 under barbiturate anesthesia (Fig. 3). Single electrical stimuli were first applied to the vicinity of the recorded neurons every 2 s, and conditioning stimuli (30 trains of five shocks) were then applied through the same electrode at the same intensity, every 2 s. For each neuron, we tested the effect of three frequencies for the conditioning trains: 10, 40 and 100 Hz. The order of the conditioning frequencies was random.

We first tested that single stimuli applied at 0.2 Hz did not induce plasticity. We found that when no conditioning protocol is applied, the amplitude of the response was unchanged throughout the whole recording (see amplitude of the response to stimulation 1 in Fig. 7). This finding is similar to what was found with callosally elicited responses in the neocortex in vivo (Cissé et al., 2004). On the other hand, conditioning trains of stimuli at various frequencies induced a change in the postsynaptic response in 77% of neurons. The change elicited by stimulation of the local cortical network consisted either of potentiation or depression, but most neurons showed a clear frequency-dependent effect of the conditioning stimuli. As shown in the neuron depicted in Figure 3B, the postsynaptic response was not affected by repetitive trains at 40 and 100 Hz, but trains at 10 Hz produced a significant decrease of the PSP amplitude. The general tendency was that depression was more prevalent than potentiation, regardless of the conditioning frequency. The percentage of neurons showing depression and potentiation was respectively of 36.0 and 20.0% for 10 Hz, 30.0 and 20% for 40 Hz, and 23.5 and 11.8% for 100 Hz conditioning frequency (Fig. 3D). Taking all conditioning frequencies together, depression was observed in 63.3% of the PSP that exhibited plastic changes. Plasticity appeared to be more effectively activated with lower frequencies. However, the difference in terms of the proportions of events showing lasting changes did not reach significance ($\chi^2 = 1.871$, $P = 0.760$): 10, 40 and 100 Hz trains induced a change in PSP amplitude in 56.0, 50.0 and 35.3% of the neurons respectively.

In seven neurons, responses to single shock stimuli were recorded for 20--60 min after application of the first conditioning protocol, which had induced depression ($n = 4$) or potentiation ($n = 3$), to assess the duration of the plasticity. The PSP amplitude was found to return to baseline after 5--31 min (16 ± 8 min, mean ± SD). Figure 4A shows an example of a neuron recorded under barbiturate anesthesia in which 10 Hz trains induced a depression that lasted for 31 min.

To test the involvement of NMDA receptors in the induction of plasticity by stimulation of the local cortical network,
we performed experiments using the same protocol in cats anesthetized with a mixture of barbiturate and ketamine-xylazine to block NMDA receptors. We found that the ketamine-induced blockade of NMDA receptors did not affect significantly the expression of plasticity in the local cortical network. Out of 27 neurons, 74.1% displayed a change in the amplitude of the PSP after conditioning protocols ($\chi^2 = 0.0580, P = 0.809$). The percentage of cells showing a change in PSP amplitude after 10, 40 and 100 Hz conditioning pulse-trains was 53.8, 47.8 and 33.3% respectively. Depression was predominant over potentiation, the percentage of neurons showing depression and potentiation being respectively 34.6 and 19.2% for 10 Hz, 30.4 and 17.4% for 40 Hz, and 22.2 and 11.1% for 100 Hz conditioning frequency. Taking all conditioning frequencies together, depression was observed in 64.5% of the PSP that exhibited plastic changes. Although each neuron showed frequency dependence for the development of plasticity (Fig. 3B), the difference in efficacy of the different frequencies did not reach significance ($\chi^2 = 1.879, P = 0.758$).

As no significant difference was observed between barbiturate without ketamine and barbiturate with ketamine, the results from the two conditions were pooled together for the rest of the study. The resulting proportions of neurons exhibiting depression, potentiation or no change after conditioning protocols is shown in the histogram in Figure 3D.

Influence of Neuronal Activity on the Expression of Plasticity

We hypothesized that the sustained neuronal activity in the cortical network in vivo might influence the expression of

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**Figure 4.** Duration of the change in PSP amplitude induced by rhythmic pulse trains. (A) Barbiturate anesthesia. The lower panel depicts the evolution of the amplitude of the PSP at the peak of the depolarization before and after 10 Hz trains were applied to the same pathway. Each value is the mean amplitude for 30 consecutive responses (± SD). Statistical difference with control (before 10 Hz) was assessed using one-way ANOVA [$F(39,1160) = 50.55, P < 0.001$] and the Tuckey-Kramer test; black-filled circles indicate statistical difference with control ($P < 0.05$). The dotted line indicates the mean amplitude before conditioning protocol. The upper panels shows averaged responses ($n = 30$) before, 19 min after and 32 min after 10 Hz trains. The dashed line indicates the baseline $V_m$. (B) Cat under ketamine-xylazine anesthesia. Lower panel, evolution of the PSP amplitude. Each value is the mean amplitude for 30 consecutive responses (± SD). Statistical difference with control (before 40 Hz) was assessed using one-way ANOVA [$F(32,957) = 5.47; P < 0.001$] and the Tuckey-Kramer test; black-filled circles indicate statistical difference with control ($P < 0.05$). The upper panels shows averaged responses ($n = 30$) before, 24 min after and 28 min after 40 Hz trains.
synaptic plasticity by maintaining cortico-cortical synapses at a given level of potentiation or depression. To test this hypothesis, we performed experiments using the same conditioning protocols in two other preparations: one, the intact cortex under ketamine-xylazine anesthesia; and the other, the small isolated cortical slab under ketamine-xylazine anesthesia. These two preparations are characterized, respectively, by a higher and a lower level of neuronal activity as compared to barbiturate anesthesia (compare panels A in Figs 5 and 6). The mean firing rate of cortical neurons recorded in the intact cortex under barbiturate (1.85 ± 1.9 Hz) was not significantly different from that of cortical neurons recorded under a mixture of barbiturate and ketamine-xylazine (1.77 ± 2.1 Hz, t-test, P = 0.89), but markedly differed from the mean firing rate of cortical neurons under ketamine-xylazine alone (6.49 ± 4.5 Hz, t-test, P < 0.001) and of neurons recorded in the isolated cortical slab (0.39 ± 0.6 Hz, t-test, P < 0.001). Thus, the steady state of synaptic plasticity should be higher in the intact cortex under ketamine-xylazine anesthesia than in the neocortical slab.

The number of neurons in which we were able to induce a change in the PSP under ketamine-xylazine anesthesia was significantly lower than under barbiturate or barbiturate plus ketamine-xylazine anesthesia (χ² = 4.940, P = 0.026). Under ketamine-xylazine anesthesia, out of 31 neurons only 51.6% showed a significant change in the PSP amplitude after at least one frequency conditioning. Figure 5B shows an example of a neuron in which no change in the PSP was observed, whether the response fell during the depolarizing phase of the slow oscillation (also called 'up' state, filled black circles) or during the hyperpolarizing phase of the slow oscillation (also referred to as 'down' state, open black circles). Another difference between barbiturate or mixed barbiturate plus ketamine-xylazine anesthesia and ketamine-xylazine alone was that, when a change in the PSP was observed, potentiation was more prevalent than depression. Overall, potentiation represented 61.1% of the changes. An example of increased PSP after 40 Hz and 100 Hz conditioning pulse-trains is shown in Figure 5C. The PSP was enhanced in both 'up' and 'down' states. The changes in PSP amplitude were also dependent on the frequency of the conditioning trains when individual cells were analyzed (see the depression after 10 Hz in Fig. 5C). Overall, the percentages of cells in which depression and potentiation were observed were, respectively, 14.8 and 22.2% with 10 Hz, 14.3 and 32.1% with 40 Hz, and 21.4 and 25.0% with 100 Hz (Fig. 5D). However, no statistical difference was observed between frequencies (χ² = 1.320, P = 0.858). The duration of plasticity was assessed in three neurons that displayed enhanced PSP amplitude and in two neurons that showed decreased PSP amplitude after conditioning. The example depicted in Figure 5.
4B shows potentiation of the PSP that was still significant 24 min after conditioning but declined thereafter. The plastic changes observed here were maintained for between 4 and 24 min (mean 13 ± 8 min) before declining to control levels.

In the isolated cortical slab under ketamine-xylazine anesthesia (Fig. 6), the percentage of neurons displaying plasticity was significantly higher than in the intact cortex under barbiturate or ketamine-xylazine anesthesia (χ² = 12.655, P = 0.0004 versus barbiturate and χ² = 24.537, P < 0.0001 versus ketamine-xylazine). We found a change in the PSP in all the neurons tested (n = 29) and in the majority of the cases we observed a marked depression of the PSP, regardless of the frequency used (depression represented 87.5% of the changes). Figure 6B,C shows two examples of cells in which trains at 40 and 100 Hz (B), and at 100, 10 and 40 Hz (C) induced a significant decrease in the PSP amplitude. High-frequency stimulation tended to be more efficient in inducing depression; a slight potentiation could be observed in a few neurons using 10 Hz, or to a lesser extent 40 Hz, conditioning trains. But no significant difference was found between the conditioning frequencies (χ² = 7.004, P = 0.136). The percentages of neurons exhibiting depression and potentiation were respectively 51.7 and 17.2% after 10 Hz, 73.7 and 5.3% after 40 Hz, and 76.5 and 0% after 100 Hz.

Specificity of the Change in PSP

A change in PSP amplitude could be due a change in V_m and/or R_in. For this reason we discarded from database those neurons showing changes in mean V_m of >3 mV. We also tested whether changes in the PSP were specific to the conditioned synaptic pathway. For this purpose, we obtained responses in the same neurons (n = 7) from stimulation via two electrodes separated by 2-3 mm. The conditioning protocol was applied via one electrode and single test stimuli applied alternating between the two electrodes. As shown in Figure 7A, in a neuron recorded under a mixture of barbiturate and ketamine-xylazine anesthesia, the PSP elicited by stimulating via the second electrode (stimulation 2, open circles) displayed a marked decrease after conditioning at 40 and 10 Hz applied to the same electrode. By contrast, the PSP elicited by the other electrode (stimulation 1, filled black circles) remained unchanged. We also tested whether the global R_in of the neuron was affected by the conditioning protocols. It was not. In six neurons, we applied hyperpolarizing current pulses of given amplitude in alternation with the single shock stimuli. As shown in the example depicted in Fig. 7B, the R_in was not affected by the conditioning protocols, even when the PSP was altered (see the increase in PSP amplitude after 40 Hz).

Finally, we tested whether the change in PSP was accompanied by a change in conductance during the response. We...
the neuron. In another neuron, hyperpolarizing current pulses and cortical stimuli were applied every two seconds alternatively. Trains of stimulation at 40 Hz induced an increase of the PSP. The drop in $\Delta R_{\infty}$ curve was observed.

Factors Influencing Cortical Plasticity

Previous studies on plasticity in the neocortex have pointed to an important role played by inhibition and postsynaptic firing in the establishment of plastic changes (Artola and Singer, 1990; Bear et al., 1992; Markram et al., 1997; Huemmke et al., 2002; Froemke et al., 2005). To understand what factors determined the plastic changes observed in the neocortical network in vivo, we analyzed the relationship between the change in PSP amplitude elicited by conditioning trains and the ratio between the excitatory and inhibitory components of the PSP, the number of AP elicited during the conditioning trains or the mean firing rate of the recorded neurons. There was only poor correlation between the change in PSP amplitude observed and the data used in Table 1. However, no general rules could be extracted. Interestingly, the way plasticity was influenced by the excitation/inhibition ratio or the mean number of AP during conditioning was different for each condition, indicating that the differences observed between the preparations were not only due to the properties of the elicited PSP in these conditions. For instance, a PSP with a dominant excitatory component ($R > 0$) showed a higher probability for potentiation under ketamine-xylazine anesthesia in the intact cortex, and a higher probability for depression in the isolated cortical slab (Table 1). We did not observe any relation between the dynamics of the PSP during the conditioning trains and the plastic change. As illustrated in Figure 10 for conditioning trains at 40 Hz in the three conditions, similar dynamics could lead to different changes and different dynamics could lead to similar changes.

Discussion

We report that rhythmic pulse-trains applied to the local cortical network in the frequency range of different oscillatory types occurring spontaneously during states of sleep and waking can induce a sustained change in PSP amplitude, which consists in either depression or potentiation. This form of synaptic plasticity was not mediated through NMDA receptors since it persisted under ketamine-xylazine anesthesia. The probability to induce plasticity and the sign of the change (potentiation or depression) were strongly affected by the background neuronal activity, namely, high levels of background neuronal activity in the cortical network induced the induction of plasticity but favored potentiation, whereas under the condition of reduced spontaneous activity depression was induced in the vast majority of the cases.
Long-term potentiation (LTP) and depression (LTD) have been induced in neo- and archicortex using diverse conditioning protocols that consisted in either tetanic or theta-burst stimulation of synaptic afferents, or pairing synaptic activation with postsynaptic depolarization or firing (reviewed in Tsumoto, 1992). Most of these experiments have been performed using slice preparations from young animals, sometimes with the addition of GABAergic antagonists. Fewer studies have investigated the establishment of long-term plasticity in the neocortex of adult animals in vivo (e.g. Sakamoto et al., 1987; Keller et al., 1990a,b; Baranyi et al., 1991). In most cases, tetanic or theta-burst stimulation, sometimes repeated for days (Chapman et al., 1998; Werk and Chapman, 2003; Froc and Racine, 2005), were necessary to produce LTP, while LTD was generally induced by low-frequency conditioning stimulation (1 Hz). In the present study, we investigated for the first time the effect on synaptic plasticity produced by low- and high-frequency stimulation within the range of natural cortical oscillatory activities (spindles, gamma rhythm, ripples). We obtained changes in PSP that persisted several minutes after the conditioning stimuli, and thus differed from short-term plasticity (Zucker and Regehr, 2002) but did not last for more than half an hour, and thus could not be fully assimilated to long-term plasticity (Tsumoto, 1992). That long-term plasticity was not found in the present study might be ascribed to the conditioning stimulation that did not produce postsynaptic depolarization strong enough because of the important inhibitory component of the PSP (Kirkwood et al., 1993). It is also possible that long-term plasticity in adult animals requires the activity of neuromodulatory systems, such as cholinergic neurons, in addition to appropriate synaptic activation (Wespatat et al., 2004). The low activity of cholinergic or other activating (histaminergic and noradrenergic) systems under anesthesia might have impaired the establishment of long-term plasticity. Another possibility is that the ongoing network activity rapidly resets the elicited plastic change or prevents its stabilization.

We found that conditioning stimulation protocols identical to those used in this study, but applied to the contralateral cortex, produced changes in amplitude of callosally elicited PSP that also lasted for several minutes and thus can be assimilated to ‘mid-term’ plasticity (Cisseé et al., 2004). However, in contrast to the plasticity observed in local (ipsilateral) cortical circuits, potentiation of the callosal PSP was found in all cases, regardless of the frequency of the conditioning trains. In addition, callosal mid-term plasticity was dependent on NMDA receptor activation, whereas blockade of NMDA receptors had little effect on the establishment of plasticity in the present study.

The difference that we found between the potentiation induced in the callosal pathway (Cisseé et al., 2004) and the mixed effects in local cortical circuits (present experiments) may be ascribed to a pathway-specific difference in NMDA receptor subunit composition (Kumar and Huguenard, 2003) or to a different balance between excitation and inhibition of the PSP. Indeed, the balance between excitation and inhibition is known to play an important role in the establishment of plasticity in the neocortex and pharmacologically blocking GABAergic inhibition supports LTP (Artola and Singer, 1990; Bear et al., 1992; Huemmeke et al., 2002). Since excitatory pyramidal cells project to the contralateral cortex, PSP elicited...
by contralateral stimulation are composed primarily of an excitatory response, sometimes followed by a polysynaptic inhibitory component (Cissé et al., 2003). On the other hand, electrical stimulation applied to local cortical network would have more chance to elicit direct inhibition by exciting local interneurons; therefore, PSP elicited by electrical stimulation in the neocortex are often a mixture of excitatory and inhibitory responses, as shown in this study. Although we found only poor correlation between the elicited plasticity and the excitation/inhibition ratio (see Ketamine--xylazine and Slab in Table 1). However, no general rules could be extracted from our data. This probably reflects the high complexity of the system we are dealing with and the wide range of factors and mechanisms leading to plasticity in the neocortex. Thus no simple rules can be found based on only one feature of the PSP or conditioning train.

Modulation of Mid-term Plasticity by the Level of Neuronal Activity

A major finding in our study is that the level of background neuronal activity strongly affected both the probability to induce plasticity and its sign (potentiation or depression). In the intact cortex under ketamine–xylazine anesthesia, which is characterized by a high level of neuronal activity, the probability to induce plasticity was the lowest, while the probability to obtain a potentiation was the highest. Conversely, in the isolated cortical slab, which was characterized by the lowest level of neuronal activity, the probability to induce plasticity...
Another factor that may explain the change in the expression of plasticity related to the neuronal activity is that the properties of cortical synapses are strongly affected by the ongoing neuronal activity in the cortical network in vivo. Several results suggest that the activity in the cortical network could markedly decrease the efficacy of the cortical synaptic transmission due to a decreased extracellular Ca\(^{2+}\) concentration (Heinemann et al., 1977; Massimini and Amzica, 2001), an increased failure rate (Crochet et al., 2005), short-term depression (Thomson, 1997; Tsodyks and Markram, 1997; Galarreta and Hestrin, 1998) or glutamate receptor desensitization (Trussell et al., 1993; Otis et al., 1996; Rozov et al., 2001). It is also possible that some homeostatic regulation of the synaptic transmission starts to occur within a few hours after the modification of the cortical activity (in our study the first recordings were typically obtained 3–4 h after the injection of anesthetic or the isolation of the cortical slab) and would up-regulate the synaptic efficacy when the background neuronal activity was decreased. In keeping with this hypothesis, we observed in a previous study that the synaptic efficacy between cortical neurons is higher in the isolated cortical slab than in the intact cortex (Crochet et al., 2005). We thus propose that the ongoing neuronal activity in the cortical network set the synaptic connections between cortical neurons at a steady level of short- and maybe long-term of depression; from this level the possibility to induce subsequent plasticity is decreased, but when it does induce plasticity, potentiation is prevalent. On the other hand, in the cortical slab, the reduced level of neuronal activity would significantly increase the synaptic efficacy and, therefore, any synaptic activation has much chance to induce depression.

In keeping with the idea that neuronal activity regulates synaptic efficacy and plasticity, we observed that receptor trafficking is modulated by intracellular Ca\(^{2+}\), which is itself strongly correlated with neuronal firing (Borgdorff and Choquet, 2002; Malinow and Malenka, 2002; Collingridge et al., 2004). In particular, a high [Ca\(^{2+}\)]\(_i\) would reduce the receptor mobility and thus favor the stability of synaptic connections.

**Functional Significance**

What is the functional significance of brain oscillations? Synchronization of discharge in the \(\beta/\gamma\) range (>20 Hz) is believed to serve response selection in the context of various cognitive functions such as binding, perceptual rivalry, attention, and short- as well as long-term memory (Singer, 1993, 1999; Miltner et al., 1999; Fries et al., 2001; Volgushev et al., 2003; Wespatat et al., 2004). On the other hand, synchronization at frequencies of <15 Hz (spindles, delta waves and slow oscillation), which are associated with SWS, may play an important role in memory consolidation (Hobson and Pace-Schott, 2002; Benington and...
Frank, 2003; Steriade and Timofeev, 2003). Our results showing that
rhythmic pulse-trains at frequencies that mimic endoge-


nous oscillations induce a sustained change in the cortical
responsiveness to local input support the idea that brain
oscillations generate an important functional reorganization of
the cortical network that might affect information processing as
well as short-term memory.

Although the form of plasticity reported here cannot account
for long-term memory traces, a role of endogenous oscillations
in long-term memory is not excluded. First, let us mention that
during waking and REM sleep, as well as during the depolarizing
phase of the slow oscillation during natural SWS, the \( V_m \)
of cortical neurons can reach levels of depolarization more favor-
able to NMDA receptors activation than under barbiturate
anesthesia, where the neurons are at hyperpolarized levels of
\( V_m \) (Steriade et al., 2001; Timofeev et al., 2001). Second, the short-
last, but sustained, non-NMDA-receptor-dependent changes in PSP we report here could affect the subsequent establishment of long-term plasticity: by transiently increasing or decreasing the PSP amplitude, the non-NMDA-receptor-
dependent form of plasticity might facilitate or inhibit the
activation of NMDA receptors involved in the generation of
long-term plasticity.

One of the most important findings of our study is that the
ability to induce plasticity as well as the direction of the
plasticity was strongly affected by the level of background
neuronal activity. In particular, the finding that a decrease in
neuronal activity results in a higher probability to induce
plasticity and a higher probability to obtain a depression of the
PSP suggests that maintaining a high level of discharge in the
cortical network insures a better stability of connectivity within
the cortical circuitry. This stability must be essential for the
conservation of acquired behaviors and memories.

Notes

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Froemke RC, Poo MM, Dan Y (2005) Spike-timing-dependent synaptic
interactions between thalamic and cortical inputs onto cortical
Galarreta M, Hestrin S (1998) Frequency-dependent synaptic depression
and the balance of excitation and inhibition in the neocortex. Nat
Neurosci 1:587–594.
Grenier F, Hestrin S (1996) Short-term plasticity of synapses in slice of
neocortex. Proc Natl Acad Sci USA 93:7428–7433.
and potassium during paroxysmal activity in the cerebral cortex of
Hirsch JC, Crepel F (1991) Blockade of NMDA receptors unmasks
a long-term depression of synaptic efficacy in rat prefrontal
Hirsch JC, Crepel F (1992) Postsynaptic calcium is necessary for the
induction of LTP and LTD of monosynaptic EPSP in prefrontal
Hobson JA, Pace-Schott EF (2002) The cognitive neuroscience of sleep:
neuronal systems, consciousness and learning. Nat Rev Neurosci
3:477–479.
Hübner R, Ghilardi MF, Massimini M, Tononi G (2004) Local sleep and
Huendermeier M, Eysel UT, Mittmann T (2002) Metabotropic glutamate
receptors mediate expression of LTP in slices of rat visual cortex. Eur
J Neurosci 15:1641–1645.
long-term potentiation in the cat motor cortex: intracellular record-
Keller A, Pavlides C, Asanuma H (1990b) Long-term potentiation in the


