Characterization of Synaptic Conductances and Integrative Properties During Electrically Induced EEG-Activated States in Neocortical Neurons In Vivo

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Rudolph, Michael, J. Guillaume Pelletier, Denis Paré, and Alain Destexhe. Characterization of synaptic conductances and integrative properties during electrically induced EEG-activated states in neocortical neurons in vivo. J Neurophysiol 94: 2805–2821, 2005. First published July 13, 2005; 10.1152/jn.01313.2004. The activation of the electroencephalogram (EEG) is paralleled with an increase in the firing rate of cortical neurons, but little is known concerning the conductance state of their membrane and its impact on their integrative properties. Here, we combined in vivo intracellular recordings with computational models to investigate EEG-activated states induced by stimulation of the brain stem ascending arousal system. Electrical stimulation of the pedonculopontine tegmental (PPT) nucleus produced long-lasting (≈20 s) periods of desynchronized EEG activity similar to the EEG of awake animals. Intracellularly, PPT stimulation locked the membrane into a depolarized state, similar to the up-states seen during sleep. During these EEG-activated states, however, the input resistance was higher than that during up-states. Conductance measurements were performed using different methods, which all indicate that EEG-activated states were associated with a synaptic activity dominated by inhibitory conductances. These results were confirmed by computational models of reconstructed pyramidal neurons constrained by the corresponding intracellular recordings. These models indicate that, during EEG-activated states, neocortical neurons are in a high-conductance state consistent with a stochastic integrative mode. The amplitude and timing of somatic excitatory postsynaptic potentials were nearly independent of the position of the synapses in dendrites, suggesting that EEG-activated states are compatible with coding paradigms involving the precise timing of synaptic events.

INTRODUCTION

In the neocortex, pyramidal cells are embedded in a very dense network, each cell receiving thousands of synaptic inputs from other neurons. In the parietal cortex of awake cats, neurons fire spontaneously at relatively high rates (1–20 Hz on average; Steriade 1978; Steriade et al. 2001). These cells are subjected to a sustained synaptic bombardment, which results in a high-conductance state characterized by highly fluctuating intracellular activity (reviewed in Destexhe et al. 2003). To circumvent the technical difficulty of recording in awake and conscious animals, one possibility is to use anesthetics that induce spontaneous periods (“up-states”), during which the electroencephalogram (EEG) is desynchronized, similar to the EEG of awake animals. Using this paradigm, it was possible to compare the same neurons during EEG-activated states and after suppressing network activity by microperfusion of tetrodotoxin (TTX) in the cortex (Destexhe and Paré 1999; Paré et al. 1998). This analysis revealed that during activated states, pyramidal neurons have a dramatically higher (about 500%) total conductance (i.e., a five times smaller input resistance, $R_{in}$) compared with the resting state after TTX. Synaptic activity was also responsible for a marked depolarization (average membrane potential of $V = -65 \pm 2$ mV, compared with $-80$ mV after TTX) and large-amplitude membrane potential ($V_{m}$) fluctuations ($\sigma_{V} = 4 \pm 2$ mV during up-states). Recordings during EEG-activated states in other preparations also reported similar depolarized states and low input resistance (Baranyi et al. 1993; Borg-Graham et al. 1998; Matsumara et al. 1988; Steriade 2001).

Although during up-states, the EEG is desynchronized and neurons display intracellular features similar to recordings obtained in awake animals (Matsumara et al. 1988; Steriade et al. 2001), the presence of anesthetics likely affects the network state. Consistent with this, intracellular recordings in nonanesthetized animals have revealed that the input resistance during periods of wakefulness is higher than that during the up-states of slow-wave sleep (Steriade et al. 2001). There is therefore a need to further characterize the conductance state of cortical neurons during EEG-activated states.

A well-known paradigm to obtain EEG-activated states during anesthesia consists in stimulating the brain stem ascending arousal system, which is believed to maintain the wake state in physiological conditions (Moruzzi and Magoun 1949). Electrical stimulation of specific structures of the brain stem or basal forebrain is known to induce periods of EEG desynchronization. This is the case of the pedonculopontine tegmental (PPT) nucleus, which participates in brain arousal in part by its cholinerergic projections to the thalamus (Paré et al. 1988; Steriade et al. 1987). The PPT also sends projections to the portions of the basal forebrain containing corticopetal cholinergic neurons (Hallanger and Wainer 1988; Semb et al. 1988; Woolf and Butcher 1986). In particular, there is a possibility that some of these projections are glutamatergic because horseradish peroxidase injections in the basal forebrain retrogradely labeled only noncholinergic cells of the PPT (Carnes et al. 1990; Steriade and Buzsaki 1990). It is therefore possible that PPT-induced EEG activation occurs through cholinergic effects in both thalamus and cortex, the latter being disynaptically caused by the basal forebrain. This duality of pathways does not seem strictly necessary, however, because PPT stimulation still evokes EEG desynchronization after large excito-
toxic lesions of either the basal forebrain (Steriade et al. 1991, 1993) or the thalamus (Steriade et al. 1993). The role of acetylcholine (ACh) in mediating the activating effect of PPT was also confirmed by its blockade by systemic administration of muscarinic antagonists, such as scopolamine (Steriade et al. 1993).

During ketamine–xylazine anesthesia, electrical stimulation of the PPT nucleus induces 10- to 30-s periods of EEG desynchronization similar to the up-states (Steriade et al. 1993). In the present paper, we used intracellular recordings of morphologically identified pyramidal neurons to study EEG-activated states evoked under ketamine–xylazine anesthesia by PPT stimulation. In combination with computational models, we estimated the conductances underlying PPT-induced states, by reference to the up-states of ketamine–xylazine anesthesia characterized previously (Destexhe and Paré 1999; Paré et al. 1998). We then used these conductance estimates to evaluate the impact of this network activity on the integrative properties of cortical neurons.

METHODS
In vivo recordings
Cortical neurons were recorded intracellularly in areas 5–7 of cats anesthetized with ketamine–xylazine. Under this anesthesia, cortical neurons display recurrent periods of activity ("up-states") similar to the awake state. Stimulating electrodes were placed in the PPT nucleus. PPT stimulation evoked periods of low-amplitude, fast-frequency EEG activity ("post-PPT states") lasting 20–30 s, during which the input resistance \( R_{in} \) and membrane potential \( V_m \) fluctuations were measured.

Experiments were conducted in agreement with ethics guidelines of the Canadian Council on Animal Care. Cats (2.5–3.5 kg) were anesthetized with a ketamine–xylazine mixture (11 and 2 mg/kg, intramuscularly). Further, lidocaine (2%) was applied to all skin incisions and pressure points. The level of anesthesia was determined by continuously monitoring the EEG ipsilateral to the intracellular recording site. Supplemental doses of ketamine–xylazine (2 and 0.3 mg/kg, respectively, intravenously [iv]) were given to maintain a recording site. Supplemental doses of ketamine–xylazine anesthesia characterized previously (Destexhe and Pare´ 1999; Pare´ et al. 1998).

Models of cortical neurons and synaptic noise

Under the assumptions that synaptic noise constitutes the main source of membrane potential fluctuations in EEG-activated states in vivo, and that other potential noise sources, such as channel noise, provide here only very little contribution (see Manwani and Koch 1999) and are thus negligible, we constructed several models to characterize cortical network activity and investigate integrative properties during such active states. These models are distinguished by their level of complexity and can be used to address different aspects of neuronal dynamics.

A first type of model, the point-conductance model (Destexhe et al. 2001), represents the membrane potential fluctuations by stochastic processes. The advantage of this representation is that synaptic activity can be characterized by a few parameters (mean conductance, variance, decay time). In addition, the \( V_m \) distribution can be assessed analytically (Rudolph and Destexhe 2003b), which enables a direct estimate of these parameters from experimental recordings (Rudolph et al. 2004; see RESULTS).

The point-conductance model consisted in a single-compartment neuron described by the passive membrane equation

\[
\frac{dV(t)}{dt} = -G_i[V(t) - E_i] - I_{in}(t) + I_{ext}(t)
\]

subject to a total synaptic current \( I_{syn}(t) = g_e(t)[V(t) - E_e] + g_i(t)[V(t) - E_i] \) and constant external (stimulating) current \( I_{ext} \). In Eq. 1, \( V(t) \) denotes the membrane potential, \( C = C_m \) is the membrane capacitance (membrane area \( a \), specific membrane capacitance \( C_m = 1 \mu F/cm^2 \)), and \( G_i = 1/R_{in} \) and \( E_i, E_e \) denote the leak conductance (input resistance \( R_{in} \)) and reversal potential, respectively. \( g_e(t) \) and \( g_i(t) \) are time-dependent global excitatory and inhibitory conductances described by one-variable stochastic processes similar to the Ornstein–Uhlenbeck process (Uhlenbeck and Ornstein 1930)

\[
\frac{dg_{e,i}(t)}{dt} = -\frac{1}{\tau_{e,i}}[g_{e,i}(t) - g_{e,i0}] + \sqrt{\frac{2\sigma_{e,i}^2}{\tau_{e,i}}} \xi(t)
\]

with respective reversal potentials \( E_e = 0 \) mV and \( E_i = -80 \) mV. These two synaptic contributions represent respectively the glutamate \( \alpha \)-amino-3-hydroxy-5-methyl-4-isoxazolepropionic (AMPA) and \( \gamma \)-aminobutyric acid type A (GABA_A) postsynaptic receptors. Here, \( g_{e0} \) and \( g_{i0} \) are the mean conductances, \( \tau_e = 2.73 \) ms and \( \tau_i = 10.49 \) ms denote the noise time constants (Destexhe et al. 2001), \( \sigma_e \) and \( \sigma_i \) are the noise SDs. \( \chi_e(t) \) and \( \chi_i(t) \) denote independent Gaussian white-noise processes of unit SD and zero mean. Values for the passive cellular properties, \( E_e \) and \( G_i \), as well as effective synaptic conductances, the means \( g_{e0} \) and \( g_{i0} \) and SDs \( \sigma_e \) and \( \sigma_i \), were estimated from experiments (see RESULTS).
In a second type of model, the one-compartment model with multiple synaptic inputs, $V_m$ fluctuations were caused by a large number of individual synaptic conductances. Despite the large number of parameters describing the kinetics of each synaptic terminal, the simple spatial structure of the model neuron allows one to treat this type of model in the context of discrete stochastic calculus (see e.g., Rice 1945). The advantage of this level of description is that it establishes a link between the dynamics of $V_m$ fluctuations and the dynamics of the activity at synaptic terminals and thus provides a framework for linking intracellular recordings with the activity of the surrounding network (Rudolph and Destexhe 2005).

We considered two-state kinetic models of AMPA and GABA_A postsynaptic receptors (Destexhe et al. 1998). The synaptic current resulting from $N_{\text{AMPA}} = 10,018$ and $N_{\text{GABA}} = 2,249$ synapses was given by

$$I_{\text{syn}}(t) = \sum_{n=1}^{N_{\text{AMPA}}} g_{\text{AMPA}} m_{\text{AMPA}}^n (t) [V(t) - E_e] + \sum_{n=1}^{N_{\text{GABA}}} g_{\text{GABA}} m_{\text{GABA}}^n (t) [V(t) - E_i]$$

where $g_{\text{AMPA}}$ and $g_{\text{GABA}}$ are the quantal conductances, and the variables $m_{\text{AMPA}}^n(t)$ and $m_{\text{GABA}}^n(t)$ represent the fractions of postsynaptic receptors in the open state at each synapse. These variables were described by the kinetic equation

$$\frac{dm_{\text{AMPA}}(t)}{dt} = \alpha_{\text{AMPA}}[1 - m_{\text{AMPA}}(t)] - \beta_{\text{AMPA}}m_{\text{AMPA}}(t)$$

Here, $[T](t)$ denotes the transmitter concentration in the synaptic cleft ($[T] = T_{\text{max}}$ for a time period $t_{\text{spike}}$ after a spike occurred and $[T] = 0$ until the next release), $\alpha_{\text{AMPA}}$ and $\beta_{\text{AMPA}}$ are forward and backward binding rate constants for excitation and inhibition. Kinetic parameters obtained by fitting the model to postsynaptic currents recorded experimentally (Destexhe et al. 1998) were $g_{\text{AMPA}} = 1.2 \text{nS}$, $g_{\text{GABA}} = 0.6 \text{nS}$, $\alpha_e = 1.1 \times 10^6 \text{M}^{-1} \text{s}^{-1}$, $\beta_e = 670 \text{s}^{-1}$ for AMPA receptors, $\alpha_i = 5 \times 10^4 \text{M}^{-1} \text{s}^{-1}$, $\beta_i = 180 \text{s}^{-1}$ for GABA_A receptors, $T_{\text{max}} = 1 \text{mM}$, and $t_{\text{spike}} = 1 \text{ms}$. To simulate synaptic background activity, all synapses were activated randomly according to independent but temporally correlated (correlation measure $c$; see Rudolph and Destexhe 2001) Poisson processes with mean rates of $\nu_{\text{exc}}$ and $\nu_{\text{inh}}$ as well as equal temporal correlation $c$ for AMPA and GABA_A synapses, respectively. Values for the temporal correlation $c$ and release rates $\nu_{\text{exc}}, \nu_{\text{inh}}$ at excitatory and inhibitory synaptic terminals, respectively, were estimated from experiments (see RESULTS). N-Methyl-D-aspartate (NMDA) receptors were not included because all experiments were obtained under ketamine–xylazine anesthesia, and ketamine is an NMDA blocker (e.g., Liu et al. 2001). GABA_B receptors were not incorporated either because no GABA_B inhibitory postsynaptic potential (IPSP) could be observed in an in vivo intracellular study of the laminar distribution of IPSPs in cat area 5–7 (Contreras et al. 1997).

A third type of model, the detailed biophysical model, is intended to simulate neuronal dynamics as faithfully as possible by incorporating a realistic distribution of numerous synaptic terminals across spatially extended active or passive dendritic structures. We used this type of model to address questions of dendritic integration as well as subthreshold and suprathreshold responses to spatiotemporally distributed synaptic inputs. The detailed biophysical model considered here consisted of a compartmental model of a morphologically reconstructed cortical pyramidal neuron from lower layer V of cat parietal cortex. The cell was stained with neurobiotin and its morphology was reconstructed with a NeuroLucida system (MicroBrightField, Williston, VT), using a ×100 objective and correction for tissue shrinkage. The three-dimensional (3D) geometry was incorporated into the NEURON simulation environment, and included a dendritic surface correction for spines (assuming that spines constitute 45% of the dendritic membrane area). The corrected membrane area of this cell was $a = 34,598 \mu m^2$. Passive properties ($E_L = -78.03 \text{mV}$ and $R_m = 62.3 \text{M}\Omega$) were estimated from intracellular recordings of the reconstructed cell (see following text) and were the same for all models of this cell.

In some simulations, voltage-dependent conductances were inserted in the soma, dendrites, and axon and were described by Hodgkin and Huxley (1952) type models. The latter model included two voltage-dependent currents, a fast Na+ current $I_{\text{Na}}$ and a delayed-rectifier K+ current $I_{\text{K_d}}$ for action potential generation (Traub and Miles 1991), and which were modified to match voltage-clamp measurements in neocortical neurons (Huguenard et al. 1988). The conductance densities used were of 8.4 and 7 mS/cm² throughout soma and dendrites, and were ten times higher in the axon. To account for the spike-frequency adaptation and afterhyperpolarization commonly observed in “regular-spiking” neurons (Connors and Gutnick 1990), a slow voltage-dependent K+ current $I_{\text{M}}$ (muscarinic potassium current; Gutfreund et al. 1995) was added to conductance densities of 0.35 mS/cm² (soma and dendrites, no $I_{\text{M}}$ in axon) was added. To test for robustness of the results, simulations with additional fast inactivating A-type K+ current $I_{\text{A}}$ (model from Migliore et al. 1999; conductance density from Bekkers 2000), T-type low-threshold Ca2+ current $I_{\text{T}}$ (model from Traub et al. 2003; conductance density from Hamill et al. 1991), hyperpolarization-activated current $I_{\text{h}}$ (model and nonuniform conductance density from Stuart and Spruston 1998), as well as the voltage-dependent cation nonelective current $I_{\text{CAN}}$ activated by muscarinic receptor stimulation (Haj-Dahane and Andrade 1996) were used. AMPA and GABA_A synaptic terminals (kinetic models as described above) were inserted into dendrites with densities as estimated from morphological studies (see DeFelipe and Farín 1992; DeFelipe et al. 2002; $N_{\text{AMPA}} = 10,018$ and $N_{\text{GABA}} = 2,249$). Details of this model can be found in previous papers (Destexhe and Paré 1999; Rudolph and Destexhe 2003a).

All simulations were performed using the NEURON simulation environment (Hines and Carnevale 1997) and were run on PC-based workstations under the LINUX operating system. Simulations were performed with a temporal resolution of 0.1 ms (detailed biophysical model) and 0.05 ms (point-conductance and one-compartment model). For each parameter set, 200 s of neural activity were simulated.

Characterization of synaptic activity

To assess synaptic activity in different EEG-activated states, various methods based on the characterization of intracellular activity were used. In all cases, intracellular activity was characterized by the mean and variance of the membrane potential obtained from Gaussian fits of $V_m$ distributions obtained at different levels of DC current injection. Passive parameters, in particular $E_L$ and the total input resistance $R_{\text{inp}}$, were obtained from linear fits of the $I$–$V$ curves. Statistically, this approach is very robust and, with the restriction to the linear regime of the $I$–$V$ curves, provided estimates whose error was <10%. Specifically, linear fits of the $I$–$V$ curve obtained from intracellular recordings during down-states yield the leak reversal potential $E_L$ for each cell. Fits to the $I$–$V$ curve obtained during up-states yield values for the input resistance $R_{\text{inp}}$ in these states. The passive input resistance $R_{\text{inp}}$ was then estimated for each cell under the assumption of a ratio $R_{\text{inp}}/R_{\text{TNX}} = 5.38$ between the input resistance in the absence of synaptic activity, equaling the desired passive input resistance, and $R_{\text{inp}}$ (Destexhe and Paré 1999). All estimated values are given as means ± SD, deduced from the statistical characterization of corresponding values for each individual cell.

A first method, referred to as the standard method, can be defined by taking the temporal average of the passive membrane equation (Eq. 1). Assuming that the average activity of the membrane potential remains constant (steady-state), the left-hand side of Eq. 1 vanishes, leading to

$$\bar{V} = E_i + r_n r_e E_e + r_e E_e$$

where $\bar{V}$ denotes the average membrane potential and $r_{\{n,e\}} = \frac{\nu_n}{\nu_n + \nu_e}$.
of a subset of seven neurons that were morphologically identified by intracellular injection of neurobiotin as layer V pyramidal cells. All of these cells showed slow spontaneous membrane potential oscillations between periods of firing activity (up-states) and silent periods (down-states; frequency between 0.2 and 1 Hz). Up- and down-states occurred synchronously with changes in EEG activity: the down-states were associated with slow waves, and the up-states were paralleled with increased gamma power in the EEG (see gray bars in Fig. 1A).

In many respects, up-states are comparable to the activity seen in the wake state (see Destexhe et al. 2003 for a review); neurons fire tonically at 1 to 20 Hz, their membrane potential is depolarized by several millivolts with respect to the resting state \((V = -72.85 \pm 10.14 \text{ mV})\) during up-states compared with \(V = -85.21 \pm 7.33 \text{ mV}\) during down-states; Fig. 2A, top) and displays large-amplitude, fast-frequency fluctuations \((\sigma_V = 2.86 \pm 0.84 \text{ mV}; \text{Fig. 2A, middle})\). Finally, the EEG is characterized by low-amplitude fast activity (Everts 1964; Steriade 2001; Fig. 1B, left).

Similar EEG-activated periods were obtained when the PPT nucleus was stimulated (Fig. 1, scheme). PPT stimulation evoked 20- to 30-s activated periods that were always paralleled with low-amplitude, high-frequency EEG activity (Fig. 1B, right). Such post-PPT states displayed electrophysiological characteristics similar to those seen in awake animals (Matsumura et al. 1988; Steriade 2001). The average membrane potential was slightly more hyperpolarized compared with the up-state \((V = -74.46 \pm 10.61 \text{ mV}; \text{Fig. 2A, top})\) and the \(V_m\) fluctuations slightly reduced \((\sigma_{V_m} = 2.28 \pm 1.36 \text{ mV}; \text{Fig. 2A, middle})\).

Previously (Destexhe and Paré 1999; Paré et al. 1998), we measured the effect of network activity on the input resistance \((R_{in})\) of cortical neurons by comparing intracellularly recorded cells under ketamine–xylazine anesthesia, before versus after microperfusion of TTX in the cortex. It was found that \(R_{in}\) was about five times higher under TTX. Here, the \(R_{in}\) was always significantly lower during the up-states \((R_{in} = 10.08 \pm 3.87 \text{ M\Omega}; \text{Fig. 2A, bottom})\) compared with the down-state \((R_{in} = 14.91 \pm 2.28 \text{ M\Omega}; \text{paired } t\text{-test}, P < 0.006)\). After PPT stimulation, the \(R_{in}\) increased significantly \((R_{in} = 18.03 \pm 4.94 \text{ M\Omega}; \text{Fig. 2A, bottom and B})\) by 44 \pm 16%, yielding the \(R_{in}\) ratio of 2.09 \pm 1.23 between up-states and post-PPT states. Assuming a fixed ratio between the \(R_{in}\) in up-states versus in the presence of TTX \((R_{in, TTX}/R_{in, up} = 5.38)\), it follows that, on average, the \(R_{in}\) in post-PPT states is about three times smaller \((R_{in, TTX}/R_{in, PPT} = 2.58 \pm 1.52)\) than when the network is silent (Fig. 2C, bottom). Note that all \(R_{in}\) estimates given here were obtained by linear fits of the \(I–V\) curves in the corresponding states, and gave values that were in good agreement with estimates obtained by injection of short current pulses.

After PPT stimulation, the SD of the membrane potential was stable for \(\pm 20\) s (Fig. 2C, top), before increasing as a result of the alternating pattern of up- and down-states (slow oscillations). In contrast, the \(R_{in}\) showed higher values only for shorter periods (around 7 s) before returning back to the values typical of up- and down-states (Fig. 2C, bottom).

**Estimation of synaptic conductances during EEG-activated states**

To estimate the respective contribution of excitatory and inhibitory conductances, we first used the standard method (see...
METHODS). We integrated the $V_m$ measurements into the expression of the passive membrane equation at steady state (Eq. 5), which yields the respective ratios of the mean inhibitory and excitatory synaptic conductances to the leak conductance (Eq. 6). Such estimates were performed for each current level in the linear $I$–$V$ regime and for each cell. An example is shown in Fig. 3A. The pooled results for all available cells indicate that the relative contribution of inhibition is severalfold greater than that of excitation (Fig. 3B). This holds for both up-states and post-PPT states, although inhibition appeared to be less pronounced for post-PPT states (paired $t$-test, $P < 0.015$; Fig. 3, B and C). Average values are $r_i = 3.71 ± 0.48$, $r_e = 0.67 ± 0.005$.

FIG. 1. Spontaneous and pedunculopontine tegmental (PPT)–induced electroencephalogram (EEG)–activated states under ketamine–xylazine anesthesia. A: cortical neuron recorded in cat parietal cortex (areas 5–7) displays up-states (gray bars) and down-states of activity that were paralleled by slow waves in the EEG. Electrical stimulation of the PPT (100 Hz for 0.1 s; see scheme) produced long periods of desynchronized EEG activity. Intracellularly, PPT stimulation induced periods characterized by a depolarized membrane potential as well as membrane potential fluctuations and discharge activity similar to that seen in the up-states. After 20–30 s, the slow waves progressively reappeared in the EEG, paralleled by the return to alternating up-/down-state patterns. B: expanded view of segments in gray shaded boxes of A.

FIG. 2. Characteristics and time evolution of intracellular activity during EEG activation. A: up-states and post-PPT states are characterized by a marked depolarization (top: average membrane potential $V_m$) and large membrane potential ($V_m$) fluctuation amplitudes (middle: $V_m$ SD $\sigma_V$). Input resistance ($R_i$) was smaller in up-states (bottom) compared with post-PPT states (for values see text). Stars mark corresponding values obtained previously (Destexhe and Paré 1999; Paré et al. 1998). B: input resistance was estimated by injecting brief current pulses (0.4 nA in the example shown at top) in the linear portion of current–voltage ($I$–$V$) relations. $R_i$ was always smaller during the up-states compared with the state induced by PPT stimulation, as indicated by the steeper slope in the $I$–$V$ plot (bottom). C: SD of membrane potential $\sigma_V$ (top) and normalized $R_i$ (bottom) as a function of time after PPT stimulation (average of 7 cells; consecutive windows of 500 and 300 ms, respectively). First point in the $\sigma_V$ graph indicates the value calculated over a long period of slow oscillations. Reference $R_i$ (bottom) was the average input resistance during up-states (gray).
0.48 for up-states, and $r_i = 1.98 \pm 1.65$, $r_e = 0.38 \pm 0.17$ for post-PPT states. From this, the ratio between the mean inhibitory and excitatory synaptic conductances can be estimated: 10.35 $\pm$ 7.99 for up-states and 5.91 $\pm$ 5.01 for post-PPT states (Fig. 3C).

To check for consistency, we used the above conductance values in the passive equation to predict the average $V_m$ using Eq. 5 in conditions of reversed inhibition (pipettes filled with 3 M KCl; measured $E_i$ of $-55$ mV). The predicted $V$ was of $-51.9$ mV, which is remarkably close to the measured value of $V = -51$ mV (Destexhe and Paré 1999; Paré et al. 1998). This analysis thus shows that for all experimental conditions (ketamine–xylazine anesthesia, PPT-induced activated states, and reversed inhibition experiments), inhibitory conductances are severalfold greater than excitatory conductances. This conclusion is also in agreement with the dominant inhibitory conductances seen in the cortex of awake cats during spontaneous activity as well as during natural sleep (Pospischil et al. 2005).

To determine the absolute values of conductances and their variance, we used a second approach, called the VmD method (Rudolph et al. 2004; see METHODS). This analysis makes use of an analytic expression of the steady-state $V_m$ distribution, given as a function of effective synaptic conductance parameters, which can be fit to experimentally obtained $V_m$ distributions. Figure 4A illustrates this method for a specific example of up-state and post-PPT state. Restricting to a linear regime of the $I$–$V$ relation (see Fig. 4A, insets), by fitting the $V_m$ distributions $p(V)$ obtained at different current levels with Gaussians (Fig. 4B, left), the mean and variance of excitatory and inhibitory synaptic conductances can be deduced (Fig. 4B, right). Because the VmD method requires two different current levels, the available experimental data for three (or four) current levels allowed three (or six) possible pairings. For each investigated cell, the values obtained from all pairings were averaged (see METHODS). In a first analysis, we estimated synaptic conductances by reference to the estimated leak conductance in the presence of TTX. This analysis yielded the following absolute values for the mean and variance of inhibitory and excitatory synaptic conductances (see Fig. 5, A and B): $g_{i0} = 70.67 \pm 45.23$ nS, $g_{e0} = 22.02 \pm 37.41$ nS, $\sigma_i = 27.83 \pm 32.76$ nS, $\sigma_e = 7.85 \pm 10.05$ nS for up-states; and $g_{i0} = 37.80 \pm 23.11$ nS, $g_{e0} = 6.41 \pm 4.03$ nS, $\sigma_i = 8.85 \pm 6.43$ nS, $\sigma_e = 3.10 \pm 1.95$ nS for post-PPT states. In agreement with the results obtained with the standard method (see above), these values show a much greater contribution of inhibitory conductances, albeit less pronounced in post-PPT states (paired $t$-test, $P < 0.07$ for ratio of inhibitory and excitatory mean, $P < 0.05$ for ratio of inhibitory and excitatory SD in both states). Ratios between inhibitory and excitatory mean conductances were $14.05 \pm 12.36$ for up-states and $9.94 \pm 10.1$ for post-PPT states (Fig. 5B, right). Moreover, inhibitory conductances displayed the greatest variance (the SD of the inhibitory synaptic conductance $\sigma_i = 4.47 \pm 2.97$ times larger than $\sigma_e$ for up-states and $3.16 \pm 2.07$ times for post-PPT states; Fig. 5B, right) and thus have a determinant influence on $V_m$ fluctuations.

**Figure 3.** Contribution of excitatory and inhibitory conductances during activated states as estimated by application of the standard method. A: representative example for estimates of the ratio between mean excitatory and leak conductance (left) as well as mean inhibitory and leak conductance (middle) in up-states (light gray) and post-PPT states (dark gray). These estimates were obtained by incorporating measurements of the average $V_m$ into the passive membrane equation (see METHODS; estimated values: $r_i = 4.18 \pm 0.01$ and $r_e = 0.20 \pm 0.01$ for up-state, $r_i = 2.65 \pm 0.09$ and $r_e = 0.28 \pm 0.09$ for post-PPT state), and yield a severalfold greater mean for inhibition than for excitation (right; $\bar{g}_i/\bar{g}_e = 20.68 \pm 1.24$ for up-state, $\bar{g}_i/\bar{g}_e = 9.81 \pm 3.23$ for post-PPT state). B: pooled results for 6 cells. In all cases, a larger inhibitory contribution was found (dashed line indicates equal contribution). C: average ratio between inhibitory and excitatory synaptic conductances was about 2 times larger for up-states (for values see text).
FIG. 4. Estimation of synaptic conductances during activated states using the VmD method. A: examples of intracellular activity during up-states (left; up-states indicated by gray bars) and post-PPT states (right). Insets: recorded cell (middle) and enlarged intracellular traces (gray boxes) as well as the I–V curves for the given cell. B: membrane potential distributions ρ(V) (left) for up-states (left) and post-PPT states (right) at 2 different injected currents \( I_{\text{ext}} = -1.04 \text{nA} \) and \( I_{\text{ext}} = 0.04 \text{nA} \). Most investigated \( V_m \) distributions were symmetric and values for the mean \( \bar{V} \) and SD \( \sigma \) of the membrane potential were obtained by Gaussian fits (black). Right: estimations of the mean of excitatory and inhibitory synaptic conductances \( (g_e) \) and \( (g_i) \) as well as their SDs \( (\sigma_e) \) and \( (\sigma_i) \) respectively. Estimated values: \( g_e = 4.13 \pm 4.29 \text{nS} \), \( g_i = 41.08 \pm 34.37 \text{nS} \), \( \sigma_e = 2.88 \pm 1.86 \text{nS} \), \( \sigma_i = 18.04 \pm 2.72 \text{nS} \), \( g_e/g_i = 21.13 \pm 16.57 \), \( \sigma_e/\sigma_i = 7.51 \pm 3.9 \) for up-state; \( g_e = 5.94 \pm 2.80 \text{nS} \), \( g_i = 29.05 \pm 22.89 \text{nS} \), \( \sigma_e = 2.11 \pm 1.15 \text{nS} \), \( \sigma_i = 7.66 \pm 7.93 \text{nS} \), \( g_e/g_i = 6.53 \pm 5.52 \), \( \sigma_e/\sigma_i = 3.06 \pm 2.09 \) for PPT-state. These estimates show a severalfold greater contribution of inhibition over excitation in both states, with a ratio between inhibitory and excitatory conductances (Krnjevic et al. 1971). However, at hyperpolarized levels, the impact of neuromodulators is expected to be small (McCormick and Prince 1986).

\[ G_{KL} \text{ and a leak potassium conductance sensitive to neuromodulators } G_{KL} \]

\[ G_L = G_{LO} + G_{KL} \]  

Moreover, denoting with \( E_R \) the potassium reversal potential, the passive leak reversal potential in the presence of \( G_{KL} \) takes the form

\[ E_L = \frac{G_{LO}E_{LO} + G_{KL}E_K}{G_{LO} + G_{KL}} \]  

where \( E_{LO} \) denotes the reversal for the \( G_{LO} \) conductance.

Introducing the scaling parameter \( \alpha (0 \leq \alpha \leq 1) \) by rewriting \( G_{KL} = \alpha G_{L2} \), the impact of the neuromodulator-sensitive leak conductance can be tested. Here, \( \alpha = 0 \) denotes the condition where the effect of neuromodulators on leak conductance is negligible, whereas for \( \alpha = 1 \), the totality of the leak is suppressed by neuromodulators.

Experiments indicate that the change of \( R_m \) of cortical neurons induced by ACh is \(<40\%\) at a depolarized \( V_m \) between \(-55 \text{ and } -45 \text{ mV} \) (39\% in Krnjevic et al. 1971; 26.4 \pm 12.9\% in McCormick and Prince 1986), and drops to about 5\% at
hyperpolarized levels between $-85$ and $-65\,\text{mV}$ ($4.6 \pm 3.8\%$ in McCormick and Prince 1986). The range of $V_m$ in our experiments corresponds in all cases to the latter values, so we would expect $\alpha$ to be small, around 0.05 to 0.1 (i.e., 5 to 10% $R_m$ change). We repeated the conductance analysis for different values of $\alpha$. Although this analysis shows that there can be up to twofold changes in the values of $g_{e0}$ and $g_{i0}$ (see Fig. 4C for a specific example and Fig. 5C for population result), for $\alpha$ between 0.05 to 0.1 these changes are minimal. Moreover, the finding that synaptic noise is mainly inhibitory in nature is not affected by incorporating the effect of ACh on $R_m$ and $E_L$ (Figs. 4C and 5C, right).

**Biophysical models of EEG-activated states**

One of the neurons recorded in this study was reconstructed using a computerized tracing system. The reconstructed 3D pyramidal morphology, shown in Fig. 6A, was integrated into the NEURON simulation environment (Hines and Carnevale 1997). The constructed model incorporated a realistic density of excitatory and inhibitory synapses, as well as quantal conductances adjusted according to previous estimates (see METHODS). The model was then compared with intracellular recordings obtained in the same cell. The parameters of synaptic background activity were varied until the model matched these recordings, by using a previously proposed search strategy that is based on matching of experimental constraints (Destexhe and Paré 1999), such as the average $V_m$ ($\bar{V}$), its variance ($\sigma_v$), and the $R_m$ (Fig. 6). This method allowed us to estimate the activity at excitatory and inhibitory synaptic terminals, such as the average release rate and temporal correlation (for an application of this method to up-states under ketamine–xylazine anesthesia, see Destexhe and Paré 1999).

In the particular neuron investigated, the post-PPT state was characterized by a value of $R_m$ that was about 3.25 times smaller compared with that estimated in a quiescent network state (corresponding to the $R_m$ decrease of about 69%; Fig. 6B, gray solid). Moreover, at rest the average $V_m$ was $\bar{V} = -69 \pm 2\,\text{mV}$ (Fig. 6C, gray solid) with SD of $\sigma_v = 1.54 \pm 0.1\,\text{mV}$ (Fig. 6D, gray solid). The optimal average rates leading to an intracellular behavior matching these measurements were $v_{inh} = 3.08 \pm 0.40\,\text{Hz}$ for GABAergic synapses with a ratio between inhibitory and excitatory release rates of about 0.165, resulting in $v_{exc} = 0.51 \pm 0.10\,\text{Hz}$ (Fig. 6, B and C, gray dashed). In addition, a weak correlation of $c = 0.25$ was necessary to match the amplitude of the $V_m$ fluctuations (Fig. 6D, star).

To test whether the estimated synaptic release rates and correlation are consistent with conductance measurements, we applied both the standard method and the VmD method to the computational model (see METHODS). Results from intracellular activity at nine different current levels from $-1$ to $1\,\text{nA}$,
yielding 36 paired recordings, were averaged. The intracellular activity (Fig. 7A) as well as the estimates for the mean and SD of synaptic conductances (Fig. 7C; estimated values: $g_{e0} = 5.03 \pm 0.20 \text{nS}$, $g_{i0} = 24.57 \pm 0.87 \text{nS}$, $\sigma_e = 2.12 \pm 0.18 \text{nS}$, $\sigma_i = 4.74 \pm 0.86 \text{nS}$) matched well the corresponding experimental measurements in the post-PPT state (Fig. 7C, compare light and dark gray; estimated values: $g_{e0} = 5.94 \pm 2.80 \text{nS}$, $g_{i0} = 29.05 \pm 22.89 \text{nS}$, $\sigma_e = 2.11 \pm 1.15 \text{nS}$, $\sigma_i = 7.66 \pm 7.93 \text{nS}$). Only in the case of $\sigma_i$ did the model yield a slight underestimation of the value deduced from experiments. This mismatch could reflect either an incomplete reconstruction or, simply, a larger error in the estimation of this parameter. Nevertheless, the ratios between the inhibitory and excitatory means ($g_{e0}/g_{i0} = 4.89 \pm 0.15$; model estimate using classical method: $g_{e0}/g_{i0} = 4.60 \pm 1.51$), as well as those between the SDs ($\sigma_e/\sigma_i = 2.26 \pm 0.53$), matched closely the results obtained by applying the VmD method to experimental data (Fig. 7C, right; $g_{e0}/g_{i0} = 6.526 \pm 5.518$, $\sigma_e/\sigma_i = 3.06 \pm 2.09$; experimental estimation using classical method: $g_{e0}/g_{i0} = 9.81 \pm 3.23$), thus cross-validating the different methods.

To obtain another, independent validation of our results, we estimated the conductances underlying synaptic activity, as well as their variances, by simulating an “ideal” voltage clamp (negligible electrode series resistance). The model was run at different command voltages (nine levels, ranging from −50 to −90 mV) using the same random seed and thus the same random activity at each clamped potential. After subtraction of the leak currents, the “effective” global synaptic conductances, $g_{leak}(t)$ and $g_{f}(t)$, as seen from a somatic electrode, were obtained (Fig. 7B, middle). The resulting conductance distributions (Fig. 7B, right) had a mean ($g_{e0} = 4.61 \pm 0.01 \text{nS}$, $g_{i0} = 28.49 \pm 0.01 \text{nS}$, $g_{e0}/g_{i0} = 6.18 \pm 0.02$) that corresponded quite well with those deduced from the experimental measurements by applying the VmD method (Fig. 7C, top). However, the voltage-clamp measurements yielded, in general, an underestimation of both $\sigma_e$ and $\sigma_i$ (Fig. 7B, right, and Fig. 7C, bottom; estimated values: $\sigma_e = 1.59 \pm 0.01 \text{nS}$, $\sigma_i = 4.03 \pm 0.01 \text{nS}$), whereas the ratio between both SDs was in good agreement with that obtained from experimental measurements (Fig. 7C, right; $\sigma_e/\sigma_i = 2.54 \pm 0.02$).

Robustness of synaptic conductance estimates

To test the robustness and applicability of the proposed method to more realistic situations with active dendrites capable of generating and conducting spikes, we incorporated voltage-dependent currents [$I_{NaT}$, $ICaT$, $IKd$, $IKf$] for spike generation, and a slow voltage-dependent $K^+$ current for spike-frequency adaptation, a hyperpolarization-activated current $I_h$, a low-threshold $Ca^{2+}$ current $I_CaP$, an A-type $K^+$ current $I_{KAT}$ (Fig. 8) as well as a voltage-dependent cation nonselective current $I_{CAN}$ (Fig. 9) with densities typical for cortical neurons; see METHODS into the detailed biophysical model. The presence of voltage-dependent ion currents yield, in general, nonlinear $I$–$V$ curves (see Figs. 8B and 9B). However, restricting to the linear regime of the $I$–$V$ curves (Figs. 8B and 9B, gray) provided synaptic conductance estimates using the VmD method (Figs. 8A and 9A, light gray), which were in good agreement with the estimates obtained with the passive model as well as experiments (Fig. 8A, diamonds and white bars, respectively). This suggests that the VmD method constitutes a robust way for estimating synaptic contributions to the membrane conductance even in situations where the membrane shows a nonlinear behavior arising from the presence of active conductances, but only if the linear portion of $I$–$V$ curves is considered.

In models with active conductances shown in Fig. 8, however, a comparison of conductance estimates obtained by applying the VmD method with those obtained from “ideal” somatic voltage-clamp simulations (dark gray) shows a systematic overestimation of both excitatory and inhibitory mean conductances ($g_{e0}$ and $g_{i0}$, respectively) as well as $\sigma_e$. This finding is in agreement with both theoretical and experimental...
results obtained in dynamic-clamp experiments performed in cortical slices (Rudolph et al. 2004). In the investigated cell, inserting active conductances for spike generation often led to the presence of a large number of "spikelets" at the soma (see Fig. 8C, arrows). The latter result from the arrival of full dendritic spikes, which fail to initiate corresponding somatic spikes. This high probability of spike failure is also linked to the incomplete morphological reconstruction of the given cell (see Fig. 6A), in particular of its distal dendrites. Because of their small and highly variable amplitude, these spikelets could not be reliably detected and were thus considered as part of the subthreshold dynamics. This, in turn, leads to skewed $V_m$ distributions, which result in the observed deviations in the conductance estimates compared with the passive model and ideal voltage-clamp situation, and in particular to an overestimation of excitatory conductances.

To evaluate the impact of a cholinergic modulation other than a $K^+$ conductance block described above, we inserted in our models the voltage-dependent cation nonselective current $I_{\text{CAN}}$ (Guérineau et al. 1995; Haj-Dahmane and Andrade 1996) with densities ranging from zero to two times the experimentally reported value of 0.02 mS/cm$^2$ (Haj-Dahmane and Andrade 1996; see Fig. 9). In this parameter regime, synaptic conductance estimates performed using the VmD method and an "ideal" somatic voltage clamp (Fig. 9A, light and dark gray, respectively) were again in good agreement with the results obtained from the corresponding experimental recordings and the passive model. Surprisingly, the estimated values for the means $g_{0e}$ and $g_{0i}$ as well as SDs $\sigma_e$ and $\sigma_i$ of excitation and inhibition, respectively, were affected only slightly by the $I_{\text{CAN}}$ conductance density, which suggests that in the subthreshold regime considered for estimating synaptic conductances, the impact of $I_{\text{CAN}}$ is negligible. This relative independence is a direct result of the activation current of $I_{\text{CAN}}$ (see Fig. 9B, inset), which takes large values only at strongly depolarized levels, resulting in a nonlinear $I-V$ relation for the membrane (Fig. 9B). Moreover, the subthreshold dynamics did not show spikelets (see above) and was nearly unaffected by the presence of $I_{\text{CAN}}$ in a physiologically relevant regime of conductance densities (Fig. 9C).

### Simplified models of EEG-activated states

To construct simplified models of cortical neurons in high-conductance states, we first analyzed the scaling structure of the power spectral density (PSD) of the $V_m$. For post-PPT
states (Fig. 10A), we found that the power spectral density $S(\nu)$ followed a frequency-scaling behavior described by

$$S(\nu) = \frac{D\tau^2}{(1 + 2\pi\nu\tau)^m}$$

where $\tau$ denotes an effective time constant, $D$ is the total spectral power at zero frequency, and $m$ is the asymptotic slope for high frequencies $\nu$. The latter is a direct indicator of the kinetics of synaptic currents (Destexhe and Rudolph 2004) and of the membrane potential (Fig. 10A). Consistent with this, the slope showed little variation as a function of the injected current (Fig. 10B, top) and of the membrane potential (Fig. 10B, bottom). It was nearly identical for up-states (slope $m = -2.44 \pm 0.31$ Hz$^{-1}$; not shown) and post-PPT states (slope $m = -2.44 \pm 0.27$ Hz$^{-1}$; see Fig. 10C). These results indicate that, in these cells, the subthreshold membrane dynamics are mainly determined by synaptic activity, less so by active membrane conductances.

We also verified that the values of synaptic time constants obtained previously (see METHODS) are consistent with the $V_m$ activity obtained experimentally. According to Destexhe and Rudolph (2004), the PSD of the $V_m$ should reflect the synaptic time constants, and the simplest expression (assuming two-state kinetic models) for the PSD of the $V_m$ in the presence of excitatory and inhibitory synaptic background activity is

$$S(\nu) = \frac{C_1}{(1 + 4\pi^2\nu^2)(1 + 4\pi^2\tau_1^2\nu^2)} + \frac{C_2}{(1 + 4\pi^2\nu^2)(1 + 4\pi^2\tau_2^2\nu^2)}$$

where $C_1$ and $C_2$ are amplitude parameters, $\tau_1$ and $\tau_2$ are the synaptic time constants, and $\tilde{\tau}_m$ denotes the effective membrane time constant in the high-conductance state. Unfortunately, not all those parameters can be extracted from a single experimental PSD. By using the values of $\tau_1 = 3$ ms and $\tau_2 = 10$ ms estimated previously (Destexhe et al. 2001), we obtained PSDs whose behavior over a large frequency range is in accord with that observed for PSDs of post-PPT states (Fig. 10A, dashed line). However, small variations (about 30%) around these values matched equally well (not shown), so the exact values of time constants cannot be estimated. The only possible conclusion is that these values of synaptic time constants are consistent with the type of $V_m$ activity recorded experimentally after PPT stimulation.

A first type of simplified model was constructed by reducing the branched dendritic morphology to a single compartment receiving the same number and type of synaptic inputs as in the detailed biophysical model (Fig. 11B). The behavior of this simplified model was compared with that of the detailed model (Fig. 11A). In both cases, the generated $V_m$ fluctuations (Fig. 11, A and B, left) had similar characteristics (Fig. 11, A and B, middle; $V = -70.48 \pm 0.31$ and $-69.40 \pm 0.25$ mV, $\sigma_V = 1.76 \pm 0.12$ and $1.77 \pm 0.07$ mV for the detailed and simpli-
fied models, respectively; corresponding experimental values: $V_{m} = -72.46 \pm 0.72$ mV, $\sigma_V = 1.76 \pm 0.26$ mV). Moreover, the PSD of both models displayed comparable frequency scaling behavior (Fig. 11, A and B, right; slope $m = -2.52$ and $m = -2.34$ for the detailed and single-compartment models, respectively; corresponding experimental value: $m = -2.44$). Finally, the $V_m$ fluctuations in the models matched quite well those of the corresponding experiments (Fig. 11D), and the power spectra deviated from the experimental spectra only at high frequencies ($\nu > 500$ Hz).

A second type of simplified model is to represent $V_m$ fluctuations by a stochastic process (see METHODS). The Ornstein–Uhlenbeck process (Uhlenbeck and Ornstein 1930) is the closest stochastic process corresponding to the type of noise generated by synapses, using exponential or two-state kinetic models (Destexhe et al. 2001). This type of stochastic process also has the advantage that the estimates of the mean and variance of synaptic conductances provided by the VmD method can directly be used as model parameters. Such an approach yielded $V_m$ fluctuations with distributions ($V = -70.01 \pm 0.30$ mV, $\sigma_V = 1.86 \pm 0.12$ mV) and power spectra (slope $m = -2.86$) similar to the experimental data (Fig. 11C; see Fig. 11D for a comparison between the different models).

Dendritic integration in EEG-activated states

Finally, we used the detailed biophysical model to investigate dendritic integration in post-PPT states. In agreement with previous results (Hö and Destexhe 2000; Rudolph and Destexhe 2003a), the reduced input resistance in high-conductance states leads to a reduction of the space constant and thus stronger passive attenuation compared with states where no synaptic activity is present (Fig. 12A, compare Post-PPT and...
Quiescent). Thus distal synapses experience a particularly severe passive filtering, and they must therefore rely on different mechanisms to have a significant influence on the soma.

Previously, it was proposed that during high-conductance states, neurons are in a fast-conducting and stochastic mode of integration (Destexhe et al. 2003). It was shown that the active properties of dendrites, combined with conductance fluctuations, establish particular dynamics rendering input efficacy less dependent of dendritic location (Rudolph and Destexhe 2003a). We investigated whether this scheme also applies to post-PPT states. To this end, we inserted sodium and potassium currents for spike generation into soma, dendrites, and axon (see METHODS). To test whether the temporal aspect of dendritic integration was affected by the high-conductance state, we stimulated subthreshold excitatory synaptic inputs impinging on dendrites (Fig. 12B) and characterized the resulting somatic excitatory postsynaptic potential.
tials (EPSPs) with respect to their time-to-peak (Fig. 12C, left) and peak height (Fig. 12C, right). As expected, the lower membrane time constant typical of high-conductance states led to faster rising EPSPs (Fig. 12B; compare inset traces for Quiescent and Post-PPT). Interestingly, both the amplitude and timing of EPSPs were weakly dependent on the site of the synaptic stimulation, in agreement with previous modeling results (Rudolph and Destexhe 2003a). Note that the reported effects were found to be robust against changes in active and passive cellular properties. Moreover, they held for a variety of cellular morphologies (Rudolph and Destexhe 2003a). Thus the fact that we reconstructed the morphology for only one cell in this study does not seem critical because these results do not depend on particular morphological details.

The mechanisms underlying this reduced location dependency were linked to the presence of synaptically evoked potentials.
dendritic spikes (Fig. 12, D and E). This was first shown in the model by testing the probability of dendritic spike initiation. In the quiescent state, there was a clear threshold for spike generation in the dendrites (Fig. 12D, gray). In contrast, during post-PPT states, the probability of dendritic spike generation increased gradually with path distance (Fig. 12D, black). Moreover, the probability was higher than zero even for stimulation amplitudes that were subthreshold in the quiescent case (Fig. 12D; compare black and gray dashed). Second, there was an enhancement of dendritic spike propagation. In post-PPT states, the response to subthreshold (Fig. 12E, bottom left) or superthreshold inputs (Fig. 12E, bottom right) was facilitated. Local dendritic spikes could also be evoked in quiescent states for large stimulus amplitudes, but they typically failed to propagate to the soma (Fig. 12E, top). These dynamics were very similar to those predicted by a previous model (Rudolph and Destexhe 2003a), although these models correspond to different conductance states.

DISCUSSION

Using a combination of intracellular recordings in vivo and computational models, we have characterized the properties of neocortical neurons during an EEG-activated state induced by PPT stimulation. Our main findings are that: 1) the $R_{\text{in}}$ of cortical neurons is higher after PPT stimulation than during the up-states seen under ketamine-xylazine anesthesia; 2) during up-states and after PPT stimulation, cortical neurons are in different network states, but both are high-conductance states dominated by inhibition; 3) this type of synaptic bombardment has a strong influence on dendritic action potential initiation and propagation, and therefore on the integrative properties of cortical pyramidal neurons. Below, we discuss the significance of these results.

Conductance measurements in EEG-activated states

To induce wakelike periods of EEG activation during anesthesia, we used a well-known paradigm, that is, stimulation of the brain stem ascending arousal system. Intracellularly, we found that PPT stimulation locks the membrane into a depolarized state giving rise to tonic firing, similar to spontaneous up-states (Fig. 1). Consistent with previous observations (Steriade et al. 1993). It may suggest that the EEG is locked into a “prolonged” up-state, but the $R_{\text{in}}$ is higher (44% on average) in the post-PPT states compared with that in up-states, suggesting that network activity differs in these states. This finding corroborates $R_{\text{in}}$ measurements in awake cats, which also showed higher $R_{\text{in}}$ compared with that in “up-states” of slow-wave sleep (Steriade et al. 2001). Thus EEG-activated states are of higher $R_{\text{in}}$ than that during up-states, and this seems to be true for both nonanesthetized animals and animals under ketamine-xylazine anesthesia.

We estimated the synaptic conductances by reference to a previous study (Destexhe and Paré 1999; Paré et al. 1998) in which, using identical methods, we compared the intracellular correlates of “up-states” and silent states induced by microperfusion of TTX in animals anesthetized with ketamine-xylazine. We estimate that, during PPT-induced EEG-activated states, the $R_{\text{in}}$ is about three times smaller than the $R_{\text{in}}$ in the absence of network activity. Decomposing this synaptic bombardment into excitatory and inhibitory components, using the standard approach based on the passive membrane equation, led to the conclusion that in all recorded cells, inhibitory conductances dominate synaptic conductances. The same conclusion was also reached by estimating the absolute value of excitatory and inhibitory conductances using the VmD method. This finding is in agreement with previous reports (Anderson et al. 2000; Borg-Graham et al. 1998; Destexhe et al. 2003; Hirsch et al. 1998) of dominant inhibitory conductances in network activity in vivo. Interestingly, the SD of the conductance was also large for inhibition, suggesting that inhibitory conductances provide a major contribution in setting the high-amplitude $V_m$ fluctuations characteristic of activated states. We therefore conclude that the synaptic bombardment of neocortical neurons during EEG-activated states is most likely determined by the dynamics of inhibitory conductances.

Contribution of cholinergic effects

However, this conclusion is based on decomposing the observed conductance change as resulting from only two synaptic conductances. Because the EEG-activated states induced by PPT stimulation are triggered by the release of ACh acting on muscarinic receptors (Steriade et al. 1993), and because muscarinic receptor stimulation blocks $K^+$ conductances in cortical neurons (McCormick 1989, 1992), as well as activates cationic conductances (Guérineau et al. 1995; Haj-Dahmane and Andrade 1996), the possible contribution of these conductance changes by ACh must be investigated. Indeed, including a significant ACh-sensitive component in the leak conductance affected the values of the conductances estimated using the VmD method (Fig. 5C). However, experimental measurements indicate that the $R_{\text{in}}$ change after ACh application in vitro is of almost 5% at the $V_m$ values considered here (between −85 and −65 mV; see McCormick 1992). Including ACh-sensitive cationic conductances also had minimal influence on conductance estimates (Fig. 9). We therefore estimate that the direct cholinergic contribution to the conductance state of cortical neurons during EEG-activated states is minimal.

This result may seem difficult to reconcile with the fact that the post-PPT state stems from fundamentally different network activity. How can ACh induce such marked changes in network activity while having relatively minor effects on single cells? A possible answer to this question is that only excitatory neurons were considered here, but ACh has very strong effects on cortical interneurons (Xiang et al. 1998), which are likely to have profound consequences on network dynamics. In agreement with this, we report here different amounts of inhibition in up-states compared with post-PPT states (see Figs. 3 and 5). We also measured dominant inhibitory conductances, further suggesting that regulation of inhibition seems the most efficient way of controlling network activity. The modest changes of excitability of pyramidal cells may also contribute through amplification by the divergence of the connectivity in the network. Taking these observations together, we suggest that ACh primarily affects network activity by acting on inhibition. Changing the overall level of inhibition induces an “active” network in which both excitation and inhibitory drives are lower, whereas the overall conductance is still dominated by inhibition, consistent with the present measurements. This
constitutes an interesting prediction to be tested by network models of cortical activated states.

Computational models

The estimated contributions of synaptic conductances were also confirmed by computational models of a morphologically reconstructed neuron from the present study, in which synapses were spatially distributed in dendrites and soma. By adjusting the parameters of synaptic bombardment to the recordings obtained in that cell, we obtained an independent estimate of the release conditions at excitatory and inhibitory synapses during EEG-activated states. Random (Poisson-distributed) release at both types of synapses at moderate rates could account for the measurements of the average $V_{m}$, $\sigma_V$, and $R_{in}$. These values were different from those estimated from anesthetized periods (Destexhe and Paré 1999), further indicating that EEG-activated states represent a different network state compared with up-states. This model also predicted a dominance of inhibitory conductances and there was a good agreement with the absolute values of the conductances obtained from the VmD method. These estimates should be corroborated with conductance measurements from intracellular recordings in awake and naturally sleeping animals.

Computational models were then used to evaluate the integrative properties of cortical neurons during EEG-activated states. Similar to a previous study (Rudolph and Destexhe 2003a), we found a pronounced voltage attenuation of passively propagated EPSPs along dendrites, but a higher propensity of EPSPs to trigger propagating dendritic spikes. As a consequence, synaptic activation evoked somatic responses whose magnitude was nearly independent of the location of the synapses in the dendrites. Because of the reduced time constant, the timing of somatic EPSPs was also nearly independent of the synaptic location, suggesting that this type of background activity is compatible with coding paradigms involving the precise timing of synaptic events.

Finally, the parameters (mean and variance) of excitatory and inhibitory conductances obtained using the VmD method could be directly incorporated into a simplified “point-conductance” model of background activity during EEG-activated states. This model reproduced all measurable electrophysiological parameters (average $V_{m}$, $\sigma_V$, and $R_{in}$) of EEG-activated states, but was two orders of magnitude faster to simulate compared with morphologically accurate biophysical models. This type of model should be useful to re-create artificial EEG-activated states in cortical neurons in slices using the dynamic-clamp technique (Chance et al. 2002; Destexhe et al. 2001; Rudolph et al. 2004), or in combination with the dendritic recordings (Williams 2004), to test experimentally the consequences of network activity on integrative properties of neocortical pyramidal neurons.

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