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Characterizing synaptic conductance fluctuations in cortical neurons and their influence on spike generation

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Abstract

Cortical neurons are subject to sustained and irregular synaptic activity which causes important fluctuations of the membrane potential ($V_m$). We review here different methods to characterize this activity and its impact on spike generation. The simplified, fluctuating point-conductance model of synaptic activity provides the starting point of a variety of methods for the analysis of intracellular $V_m$ recordings. In this model, the synaptic excitatory and inhibitory conductances are described by Gaussian-distributed stochastic variables, or "colored conductance noise". The matching of experimentally recorded $V_m$ distributions to an invertible theoretical expression derived from the model allows the extraction of parameters characterizing the synaptic conductance distributions. This analysis can be complemented by the matching of experimental $V_m$ power spectral densities (PSDs) to a theoretical template, even though the unexpected scaling properties of experimental PSDs limit the precision of this latter approach. Building on this stochastic characterization of synaptic activity, we also propose methods to qualitatively and quantitatively evaluate spike-triggered averages of synaptic time-courses preceding spikes. This analysis points to an essential role for synaptic conductance variance in determining spike times. The presented methods are evaluated using controlled conductance injection in cortical neurons in vitro with the dynamic-clamp technique. We review their applications to the analysis of in vivo intracellular recordings in cat association cortex, which suggest a predominant role for inhibition in determining both sub- and supra-threshold dynamics of cortical neurons embedded in active networks.

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1. Introduction

Cerebral cortical networks can generate states of intense and irregular activity, which are characterized by low-amplitude "desynchronized" fast activity in the electroencephalogram (EEG), a defining feature of the awake state. Intracellular measurements in awake animals (Woody and Gruen, 1978; Matsumura et al., 1988; Baranyi et al., 1993; Steriade et al., 2001; Timofeev et al., 2001; Rudolph et al., 2007) have shown that cortical neurons are depolarized (about $V_m \approx -60$ mV), have a low input resistance, their membrane potential ($V_m$) fluctuates, and they fire irregularly and sustainedly. During slow-wave sleep, or under several types of anesthetics (such as urethane or ketamine-xylazine), the $V_m$ displays "up-" (depolarized) and "down-" (hyperpolarized) states, which are paralleled with EEG slow waves (Metherate and Ashe, 1993; Steriade et al., 1993, 2001; Timofeev et al., 2001). During the up-state, the EEG is desynchronized and the $V_m$ of cortical neurons is depolarized and highly fluctuating, similar to the sustained activity found in awake animals (Destexhe et al., 2007). Up- and down-states have also been found in ferret cortical slices using high-potassium and low-calcium extracellular media (Sanchez-Vives and McCormick, 2000) and in rat entorhinal slices as a function of the metabolic state (Cunningham et al., 2006): these experiments indicate that intracortical circuits are able to generate such states, presumably through recurrent excitation and inhibition. TTX block of action potentials in vivo (Paré et al., 1998) and CNQX block of excitatory synapses in vitro (Cunningham et al., 2006) abolish the depolarized, fluctuating states, which confirms their synaptic origin.

The recurrent activity of cortical networks has been investigated using computational models at different levels and
2. Methods

2.1. Computational methods

A fluctuating point-conductance model driven by Poisson spike trains, in terms of models that are relatively simple, but contain variables and cannot be obtained from any single experiment, which componentation of all those models requires a large amount of information (Destexhe and Pare, 1999; Rudolph and Destexhe, 2001). Neuron responsiveness and integrative properties (Bernander et al., 1994; van Vreeswijk and Sompolinsky, 1996; Roxin et al., 2005; Barak and Tsodyks, 2007). At the single neuron level, detailed models assess the impact of massive input from the cortical ion channels and distributed synaptic inputs: studies of such models can include the cell’s morphology, a variety of intrinsic and extrinsic chaos (van Vreeswijk and Sompolinsky, 1996; Roxin et al., 2005) .

2.2. Biological preparation

In vitro experiments were performed on 380–400 m in vitro pentobarbital-anesthetized adult ferret (Marshall Europe, Lyon) thick coronal or sagittal slices from the lateral portions of the neocortex. Slices were maintained in an interface style recording chamber at 33–35 °C in slice solution containing (in mM) 124 NaCl, 2.5 KCl, 1.2 MgSO4, 1.25 NaHCO3, and 10 dextrose and aerated with 95% O2–5% CO2 to approximately 1 h, the solution was modified to contain 1 mM MgSO4, 1 mM CaCl2 and 3.5 mM KCl (Sanchez-Vives et al., 2005). We present here techniques of various degrees of biological realism and unit standard deviation. The inhibitory conductance was represented by only two stochastic conductance variables, modeled as Ornstein–Uhlenbeck (Brownian-motion-like) processes. This fluctuating point-conductance model, which contains 

\[ C \frac{V}{t} = -g_L(V - E_L) - g(V - E) - g(V - E) + I, \]

where \( C \) and \( g \) are conductances, \( E \) is the reversal potential, \( i \) is the noise source, and \( \xi \) is a Gaussian white noise source with zero mean and variance \( \sigma^2 \).

\[ \frac{g(t)}{t} = -\frac{1}{\tau} g(t) - g_0 + \sqrt{\frac{2\sigma^2}{\tau}} \xi(t), \]
2.3. Electrophysiology

2.4.1. VmD analysis

\[ I(t) = -g(V - E) - g(V - E). \]

2.4.2. Power spectral density (PSD) analysis

2.4.3. Spike-triggered average (STA) analysis

2.4. Data analysis
3. Results

3.1. The VmD method for extracting synaptic conductance parameters

3.1.1. Outline of the VmD method

\[
g(t) = \sigma^2 \left[ 1 + k \frac{(t - t_0)}{\tau^2} \right],
\]

where \( \sigma^2 \) quantifies the variance of the conductance fluctuations observed in vivo, while \( \tau^2 \) is the time constant of the conductance fluctuations. The conductance \( g(t) \) is the product of \( \sigma^2 \) and the average baseline conductance \( g_{e0} \), plus a term involving the conductance fluctuations observed in vivo.

In the VmD method, the conductance fluctuations are described by a Gaussian model, which assumes that the synaptic conductances are Gaussian-distributed stochastic variables. The model describes the evolution of the subthreshold conductance (see Section 3).
One main advantage of this Gaussian approximation is that it can be inverted, which leads to expressions of the synaptic noise parameters as a function of the \(V_m\) measurements, \(\bar{V}\) and \(\sigma_V\). By fixing the values of \(\tau_e\) and \(\tau_i\), which are related to the decay time of synaptic currents and can be estimated from voltage-clamp data and/or current-clamp by using power spectral analysis (see Section 3.2), we remain with four parameters to estimate: the means (\(g_{e0}, g_{i0}\)) and standard deviations (\(\sigma_e, \sigma_i\)) of excitatory and inhibitory synaptic conductances. To extract these four conductance parameters from the membrane probability distribution, Eq. (7) is, however, insufficient because it is characterized by only two parameters (\(\bar{V}, \sigma_V\)). To solve this problem, one possibility is to consider two \(V_m\) distributions obtained at two different constant levels of injected current \(I_{ext1}\) and \(I_{ext2}\). In this case, the Gaussian approximation (Eq. (7)) of the two distributions gives two mean \(V_m\) values, \(\bar{V}_1\) and \(\bar{V}_2\), and two standard deviation values, \(\sigma_V_1\) and \(\sigma_V_2\). The resulting system of four equations relating \(V_m\) parameters with conductance parameters can now be solved for four unknowns:

\[
\begin{align*}
\tau_e \cdot V_1 - \tau_i \cdot V_2 &= (S_{g_e} - S_{g_i}) \cdot \bar{V}_1 - (k_m - k_i) \cdot \bar{V}_2 + I_{ext1} - I_{ext2}, \\
\bar{V}_1 - \bar{V}_2 &= \left(\frac{I_{ext1} - I_{ext2}}{\sigma_V_1^2 - \sigma_V_2^2}\right) \cdot \left(\frac{\sigma_V_2^2}{\sigma_V_1^2}\right) \cdot \left(\frac{\sigma_V_1^2}{\sigma_V_2^2}\right) \\
\end{align*}
\]

\[\begin{align*}
\sigma_V_1^2 &= \frac{2C(I_{ext1} - I_{ext2})\sigma_V_1^2(E_{I_{ext1}} - V_1)^2 - \sigma_V_2^2(E_{I_{ext1}} - V_2)^2}{\tau_{i,1} (E - V_1)(E - V_2) + (E - V_2)(E - V_1)(E_{I_{ext1}} - E_{I_{ext2}})(V_1 - V_2)^2} \\
\sigma_V_2^2 &= \frac{2C(I_{ext1} - I_{ext2})\sigma_V_2^2(E_{I_{ext2}} - V_2)^2 - \sigma_V_1^2(E_{I_{ext2}} - V_1)^2}{\tau_{i,2} (E - V_1)(E - V_2) + (E - V_2)(E - V_1)(E_{I_{ext1}} - E_{I_{ext2}})(V_1 - V_2)^2},
\end{align*}\]
Fig. 2. VmD estimation of conductances from intracellular recordings in awake and naturally sleeping cats. (A) Intracellular recordings in awake and naturally sleeping (SWS) cats. Recordings were made in association cortex (area 5–7). (B) Examples of Vm distributions computed during wakefulness (Awake) and slow-wave-sleep up-states (SWS). The continuous lines show Gaussian fits of the experimental distributions. Insets: current–voltage relations obtained for these particular neurons. (C) Conductance values estimated using the VmD method. Results for the means ($g_{e0}$, $g_{i0}$) and standard deviations ($\sigma_e$, $\sigma_i$) of excitatory and inhibitory conductances, respectively, as well as their ratios are shown (error bars: standard deviations obtained by repeating the analysis using different pairs of injected current levels). (D) Grouped data showing the means and standard deviations of the conductances for different cells across different behavioral states (REM = Rapid Eye Movement sleep). Figure modified from Rudolph et al. (2007).

Here, $\tilde{\tau}\{e, i\}$ are effective time constants given by:

$$\tilde{\tau}\{e, i\} = \frac{2}{\tau\{e, i\} + \tau_m},$$

(10)

where $\tau_m = C/(G_L + g_0 + g_0)$ is the effective membrane time constant (see details in Rudolph and Destexhe, 2005; see also Richardson, 2004). These relations enable us to estimate global characteristics of network activity, such as mean excitatory ($g_{e0}$) and inhibitory ($g_{i0}$) synaptic conductances, as well as their respective variances ($\sigma_e^2$, $\sigma_i^2$), from the sole knowledge of the Vm distributions obtained at two different levels of injected current. This VmD method was tested using computational models and dynamic-clamp experiments (Rudolph et al., 2004) and was also used to extract conductances from different experimental...
3.1.2. Testing the VmD method with dynamic-clamp

The VmD method was then applied to analyze intracellular recordings of cortical neurons in vivo (Paré et al., 1998; Destexhe and Paré, 1999) were used for the analysis of the continuous fluctuations following PPT stimulation. In both cases, values obtained from previous work were used further, and recently we showed that the power spectral density (PSD) of the Vm fluctuations described by this model can be well approximated by the following expression (Destexhe et al., 2000): $\omega(t) = \frac{\mu}{\mu^2 + \omega_0^2}$.

3.1.3. Analysis of intracellular recordings of cortical neurons in vivo

The second study shows similar results across the natural sleeping and awake cats (Rudolph et al., 2007). In a first test (in 5 neurons), we performed tests of the VmD method (Rudolph et al., 2004; Piwkowska et al., 2005). In a second test, we injected fluctuating conductances (ge0, ei0, i0, e, i) into the cell from the in vitro model. We also re-estimated known parameters of synaptic conductances together with their variances decreased in the wake state compared to slow-wave-sleep up-states. In addition, especially during slow-wave-sleep up-states, inhibition dominated (Piwkowska et al., 2005). In a first test (in 5 neurons), we used the estimated parameters to inject fluctuating conductances in dynamic-clamp in the same cell, during down-states. Fig 1C compared to slow-wave-sleep up-states. In addition, especially during slow-wave-sleep up-states, inhibition dominated (Piwkowska et al., 2005). In a second test, we confirmed that the up-state recreated in dynamic-clamp. We confirmed that the up-state recreated in dynamic-clamp. We confirmed that the up-state recreated in dynamic-clamp.
3.2.3. PSD analysis of dynamic-clamp injection in real neurons is consistent not only with models, but also with conductance numerically was nearly perfect, as shown in Fig. 3A and as matching between the analytic expression and the PSD obtained of the conductance variances with the VmD method. As those Eqs. (11) or (12), both of which provided equally good fits (not noise becomes important. The template used was according to the theoretical expression of the conductance fluctuations resulting from real synaptic activity, during up- in vitro conductance injection in cortical neurons and Rudolph, 2004): SV (ω) = ...

2004). S

3.3. Estimating spike-triggering conductance configurations

Indeed, an infinite number of combinations of V

inhibitory conductances are of comparable magnitude (Fig. 4A, but in this case, inhibition has to be augmented several-fold to bombardment. Nevertheless, including the values of the method to estimate

3.2.2. Testing synaptic time constants estimates with dynamic-clamp

2005, 2007; Rudolph et al., 2005a). In this case, the scaling of high-resolution electrode compensation technique, Brette et al., considering that fluctuations around the obtained mean voltage total membrane conductance, ˜G

τ = 10 ms provided acceptable fits to the low-frequency part of the spectrum (Fig. 3C and D, red curves). In this case, however, it is apparent that the experimental PSDs cannot be fitted with the theoretical prediction (Fig. 3B): the theoretical template is used to provide estimates of the parameters SV (ω) = ...

j w , w , T , w T , w V , w , V . (11) (12) .

3.3. PSD analysis of Vm fluctuations in vitro and in vivo in vitro ( . ) w k in vivo ( . ) . w , w S - ( . ) . T , w 1 / f


400 H , w T w w k - 50 w w k . T w w , w S k . T w , w . 4 . - k .

400 H , w T w w k - 50 w w k . T w w , w S k . T w , w . 4 . - k .


Fig. 3. Fit of the synaptic time constants to the power spectrum of the membrane potential. (A) Comparison between the analytic prediction (Eq. (11); red) and the PSD of the $V_m$ for a single-compartment model (Eq. (1); black) subject to excitatory and inhibitory fluctuating conductances (Eqs. (2) and (3); $\tau_e = 3$ ms and $\tau_i = 10$ ms). (B) PSD of the $V_m$ activity in a guinea-pig visual cortex neuron (black), where the same model of fluctuating conductances as in (A) was injected using dynamic-clamp. The red curve shows the analytic prediction using the same parameters as the injected conductances ($\tau_e = 2.7$ ms and $\tau_i = 10.5$ ms). (C) PSD of $V_m$ activity obtained in a ferret visual cortex neuron (black) during spontaneously occurring up-states. The PSD was computed by averaging PSDs calculated for each up-state. The red curve shows the best fit of the analytic expression with $\tau_e = 3$ ms and $\tau_i = 10$ ms. (D) PSD of $V_m$ activity recorded in cat association cortex during activated states in vivo. The red curve shows the best fit obtained with $\tau_e = 3$ ms and $\tau_i = 10$ ms. Panel A modified from Destexhe and Rudolph (2004); panel D modified from Rudolph et al. (2005b).

Decrease necessarily comes from a similar decrease of inhibitory conductance, which is, in this case, stronger than the increase of excitatory conductance ($g_i$ curve in Fig. 4 B, right). Thus, in such states the spike seems primarily caused by a drop of inhibition.

This pattern was seen not only in the average, but also at the level of single spikes. Using a vector representation to display the conductance variation preceding spikes (each vector links the conductance state in a window of 30–40 ms before the spike with that in the 10 ms preceding the spike) shows that the majority of spikes follow the average pattern (Fig. 4C). The same features were also present when the integrate-and-fire model was used (not shown), and thus do not seem to depend on the spike generating mechanisms.

These patterns of conductance variations preceding spikes were also investigated in real neurons by using dynamic-clamp experiments to inject fluctuating conductances in vitro. In this case, performing the same analysis as above revealed similar features: spike-triggered averages (STAs) of the injected conductances displayed either increase or decrease in total conductance, depending on the conductance parameters used (Fig. 5 A), and the vector representations were also similar.
Fig. 4. Comparison between equal conductances and inhibition-dominated states in a computational model. (A) Equal conductance (left; \( g_{e0} = g_{i0} = 10 \text{nS} \), \( \sigma_\text{e} = \sigma_\text{i} = 2.5 \text{nS} \)) and inhibition-dominated states (right; \( g_{e0} = 25 \text{nS} \), \( g_{i0} = 100 \text{nS} \), \( \sigma_\text{e} = 7 \text{nS} \) and \( \sigma_\text{i} = 28 \text{nS} \)) in the point-conductance model. Excitatory and inhibitory conductances, and the membrane potential, are shown from top to bottom. Action potentials (truncated here) were described by Hodgkin–Huxley type model (Destexhe et al., 2001; Eq. (4)). (B) Average conductance patterns triggering spikes. Spike-triggered averages (STAs) of excitatory, inhibitory and total conductance were computed in a window of 50 ms before the spike. (C) Vector representation showing the variation of synaptic conductances preceding each spike. The excitatory and inhibitory conductances were averaged in two windows of 30–40 ms and 0–10 ms (circle) before the spike, and a vector was drawn between the obtained values. (Fig. 5B). It suggested that these features are independent of the spike generating mechanism but rather are caused by sub-threshold \( V_\text{m} \) dynamics.

3.3.2. A geometrical interpretation based on the point-conductance model

The configuration of synaptic conductances just before spikes can be explained qualitatively by considering that the total current must be positive at spike time, i.e.,

\[
\frac{g_{e}(E_e - V_t) + g_i(E_i - V_t) + G_L(E_L - V_t)}{\sigma_\text{e}^2 + \sigma_\text{i}^2} > 0,
\]

where \( V_t \) is the spike threshold (using an integrate-and-fire approximation). This inequality defines a half-plane in which \((g_e, g_i)\) must lie at spike time. Fig. 6A shows graphically how this inequality affects the synaptic conductances. The variable \((g_e, g_i)\) is normally distributed, so that isoprobability curves are ellipses in the plane (plotted in red). In that plane, the line \( \{g_e + g_i = g_{e0} + g_{i0}\} \) going through the center of the ellipses defines the points for which the total conductance equals the mean conductance, and the line \( \{g_e(E_e - V_t) + g_i(E_i - V_t) + G_L(E_L - V_t) = 0\} \) defines the border of the half-plane in which conductances lie at spike time. In the equal conductances regime (Fig. 6A, left), synaptic conductances are small and have similar variances, so that isoprobability curves are circular; the intersection of the half-plane with those circles is mostly above the mean total conductance line, so that the total conductance is higher than average at spike time.

3.3.2. A geometrical interpretation based on the point-conductance model

\[
T \left\{ g_e(E_e - V_t) + g_i(E_i - V_t) + G_L(E_L - V_t) = 0 \right\} > 0, \quad g_e = g_i = 10 \text{nS}, \quad \sigma = 2.5 \text{nS}. \]

\[
\begin{align*}
\sigma &= 2.5 \text{nS}, \\
g_e &= 25 \text{nS}, \\
g_i &= 100 \text{nS}, \\
\sigma_e &= 7 \text{nS}, \\
\sigma_i &= 28 \text{nS}. 
\end{align*}
\]
Fig. 5. Average conductance patterns triggering spikes in dynamic-clamp experiments. (A) Spike-triggered averages of excitatory, inhibitory and total conductance in a window of 50 ms before the spike in a cortical neuron subject to fluctuating conductance injection. The two states, equal conductances (left) and inhibition-dominated (right), were recreated similar to the model of Fig. 4. Conductance STAs showed qualitatively similar patterns. (B) Vector representation showing the variation of synaptic conductances preceding each spike (as in Fig. 4C).

In the inhibition-dominated regime (Fig. 6A, right), synaptic conductances are large and the variance of $g_i$ is larger than the variance of $g_e$, so that isoprobability curves are vertically elongated ellipses; the intersection of the half-plane with those ellipses is essentially below the mean total conductance line, so that the total conductance is lower than average at spike time.

More precisely, when isoprobability curves are circular (equal variances), then the expected total conductance is unchanged at spike time when the lines

$$\{g_e(E_e - V) + g_i(E_i - V) + G_L(E_L - V) = 0\}$$

and

$$\{g_e + g_i = g_0 + g_0\}$$

are orthogonal, i.e., when

$$E_e - V + E_i - V = 0.$$ Spikes are associated with increases in conductance when the first line has a higher slope, i.e., when

$$E_e - V > V - E_i$$

(which is typically the case).

When isoprobability curves are not circular, we can look at the graph in the space $(g_e/\sigma_e, g_i/\sigma_i)$ where isoprobability curves are circular. Then the orthogonality condition between the lines

$$\{g_e \sigma_e (E_e - V) + g_i \sigma_i (E_i - V) + G_L \sigma_L (E_L - V) = 0\}$$

and

$$\{g_e \sigma_e + g_i \sigma_i = g_0 \sigma + g_0 \sigma\}$$

reads

$$\sigma_e^2 (E_e - V) + \sigma_i^2 (E_i - V) = 0.$$ It follows that spikes are associated with increases in total conductance when the following condition is met:

$$\frac{\sigma_e}{\sigma_i} > \sqrt{\frac{V - E_i}{E_e - V}}.$$ One can also recover this result by calculating the expectation of the conductance change conditionally to the fact that the current at spike threshold is positive (implicitly, we are neglecting the correlation time constants of the synaptic conductances). Using typical values ($V_t = -55 \text{ mV}$, $E_e = 0 \text{ mV}$, $E_i = -75 \text{ mV}$), we conclude that spikes are associated with increases in total conductance when

$$\sigma_e > 0.$$ This inequality is indeed satisfied in the equal conductances regime and not in the inhibition-dominated regime investigated above.

3.3.3. Testing the geometrical prediction with dynamic-clamp

The geometrical reasoning predicts that the sign of the total synaptic conductance change triggering spikes depends only on the ratio of synaptic variances, and not on the average conductances. We have systematically tested this prediction using dynamic-clamp injection of fluctuating conductances in vitro. In 8 regular spiking cortical neurons, we scanned different parameter regimes in a total of 36 fluctuating conductance injections. Fig. 6B shows two examples from the same cell: both correspond to an average “high conductance” regime, dominated by
Fig. 6. Geometrical interpretation of the average conductance patterns preceding spikes and test in dynamic-clamp. (A) Red ellipses: isoprobable conductance configurations. Green area: conductance configurations for which the total synaptic current is positive at spike threshold. The vector representations of Figs. 4 and 5 are schematized here, and compared to the lines defined by
\[
\{ g_e(E_e - V_t) + g_i(E_i - V_t) + G_l(E_l - V_t) = 0 \} \quad \text{(solid grey line)}
\]
and
\[
\{ g_e + g_i = g_{e0} + g_{i0} \} \quad \text{(dashed grey line)}.
\]
The angle between the two lines and the aspect ratio of the ellipse determine whether spikes are preceded, on average, by total conductance increase (left) or decrease (right) (see text for further explanations). (B) Spike-triggered average conductances obtained in dynamic-clamp, illustrating that for the same average conductances, the variances determine whether spikes are preceded by total conductance increase (left) or decrease (right). (C) Geometrical prediction tested in dynamic-clamp (left): grouped data showing total conductance change preceding spikes as a function of the ratio $\sigma_e/\sigma_i$. The dashed line ($\sigma_e/\sigma_i = 0.6$) visualizes the predicted value separating total conductance increase cases from total conductance decrease cases. In addition (right), dynamic-clamp data indicates that the amplitude of change of each of the conductances before a spike is linearly correlated with the standard deviation parameter used for this conductance.

In addition, (Fig. 6C, right), the dynamic-clamp data shows that the average amplitude of change ($\Delta_1g_e = g_{e0}$, see Section 2) of each synaptic conductance preceding a spike is related, in a linear way, to the standard deviation of this conductance. For a fixed value of standard deviation, there was no significant influence of the average conductance (not shown). This observation...
\[
\tau = \tau \cdot V \cdot (w \cdot \tau). \quad (1)
\]

\[
\xi^k = 1 \sigma \sqrt{2 \Delta t} \left( g^{k+1} - g^k \left( 1 - \frac{\Delta t}{\tau} \right) - \frac{\Delta t}{\tau} g^0 \right). \quad (14)
\]

\[
\xi^k = 1 \sigma \sqrt{2 \Delta t} \left( g^{k+1} - g^k \left( 1 - \frac{\Delta t}{\tau} \right) - \frac{\Delta t}{\tau} g^0 \right). \quad (15)
\]

\[
p^k = p(g^{k+1}, g^{k+1}, g^k) = \frac{1}{2\pi} \cdot -\left( \frac{1}{\sqrt{4\Delta t}} \right)^2. \quad (1)
\]

\[
\Delta t = \tau \cdot \sigma \left( g^{k+1} - g^k \left( 1 - \frac{\Delta t}{\tau} \right) - \frac{\Delta t}{\tau} g^0 \right)^2 + \frac{\tau}{\sigma} \left( g^{k+1} - g^k \left( 1 - \frac{\Delta t}{\tau} \right) - \frac{\Delta t}{\tau} g^0 \right)^2. \quad (1)
\]
Fig. 7. Spike-triggered conductance extraction from intracellular recordings of the $V_m$ and test in dynamic-clamp. (A) Left: spike-triggered average (STA) of the $V_m$ in an integrate-and-fire extension of the point-conductance model (top trace). The numerically obtained conductance STAs (orange, green) are compared to the conductance STAs extracted from the $V_m$ (black) (bottom trace). Right: test of the STA method using dynamic-clamp. The STA of the $V_m$ is obtained following injection of fluctuating conductances (top trace). The measured conductance STAs (orange, green) are compared to the conductance STAs extracted from the $V_m$ (black) (bottom trace). (B) Grouped data comparing conductance STAs extracted using the method (est.) with the conductance STAs measured (data) following dynamic-clamp injection: amplitude of conductance change preceding the spike (top graphs) and time constant of this change (bottom graphs), for both excitation and inhibition. (C) Top: correlation between errors for excitation and inhibition on the absolute value of conductance variation. Bottom: total conductance change preceding spikes; comparison between extracted and measured STAs. Dashed lines: $Y = X$.

Centered at 0 during stimulus-evoked responses (“delayed inhibition”; see Wehr and Zador, 2003; Wilent and Contreras, 2005), we allow a non-zero delay $d$: for a positive parameter $d$, the inhibitory channel receives the input that the excitatory channel received $d$ time steps before. Eqs. (20) and (21) can be solved for $\xi_k^{1}$ and $\xi_k^{2}$, thus replacing Eqs. (14) and (15). It is then possible to proceed as in the uncorrelated case, where now, due to the delay, the matrix describing Eq. (19) has additional subdiagonal entries.

However, the application of this extended method requires the estimation of the usual leak parameters, of conductance distribution parameters—which the VmD method cannot be directly used in its current form since it is based on uncorrelated noise sources—as well as knowledge of the parameters $c$ and $d$. At present, we can only speculate on how $c$ and $d$ could be evaluated in experiments: extracellularly recorded spike trains could perhaps be used to this end, provided that simultaneously recorded single units could be classified as excitatory or inhibitory. Alternatively, different plausible $c$ and $d$ values could be scanned to examine how they could potentially influence the conductance STAs extracted from a given $V_m$ STA.
3.4.1. Testing STA estimation with dynamic-clamp

We have investigated the dependency of the estimate of the synaptic conductance parameters estimated with the VmD method (see above) on the average firing rate (for the rates up to 316 Hz). The VmD method specifically, we assumed that conductance distribution in Fig. 7A, right panel. Since we intended to evaluate the STA conductance STAs obtained directly by averaging conductance traces, since a good match was observed between the two (see one example in Fig. 7B, top). This dependency actually ensures that the error on the estimate of the synaptic conductance points to a more complex origin for the observed errors. Moreover, the errors on the amplitude of correlation between the two error measures in the case of slight too high amplitude of conductance change. The lack of correlation between the two error measures in the case of slight too fast rise of the excitatory conductance results in a severe contamination of the analyses by intrinsic conductances.

3.4.2. STA analysis of intracellular recordings of cortical neurons in vivo

The conductance STA estimation method was used to evaluate the accuracy of the conductance STA estimation method specifically, we assumed that conductance distribution in Fig. 7A, right panel. Since we intended to evaluate the STA conductance STAs obtained directly by averaging conductance traces, since a good match was observed between the two (see one example in Fig. 7B, top). This dependency actually ensures that the error on the estimate of the synaptic conductance points to a more complex origin for the observed errors. Moreover, the errors on the amplitude of correlation between the two error measures in the case of slight too high amplitude of conductance change. The lack of correlation between the two error measures in the case of slight too fast rise of the excitatory conductance results in a severe contamination of the analyses by intrinsic conductances.
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Fig. 8. Analysis of the average conductance patterns preceding spikes, same analysis as Fig. 6 B and C, but using conductance STAs extracted from the 
$V_m$, instead of the measured ones. (A) Example conductance STAs extracted from the 
$V_m$ STAs of the same cell and the same conductance injections as Fig. 6 B. (B) Left: test 
of the geometrical prediction (dashed line) using conductance STAs extracted from the 
$V_m$, and showing total conductance change preceding spikes as a function of 
the ratio $\sigma_e/\sigma_i$ (as in Fig. 6 C, left). Right: correlation between the amplitude of change of each conductance preceding a spike, as extracted from the 
$V_m$, and the 
standard deviation parameter for this conductance (compare to Fig. 6 C, right).

determine whether, on average, the total conductance increases 
or decreases prior to the spike. This ratio is independent of the 
leak conductance, but it depends on the capacitance $C$. However, 
it appears in both the numerator and the denominator of the ratio. 
Fig. 9C shows the dependency of $\sigma_e/\sigma_i$ on this parameter for 
different values of total input resistance and two sets of realistic 
values for the $V_m$ distribution parameters. This analysis indicates 
that a reasonable error on $C$ produces a limited error on the ratio $\sigma_e/\sigma_i$, and suggests that conclusions drawn from the 
in vivo data 
about the respective contributions of excitation and inhibition in 
triggering spikes are valid.

4. Discussion

4.1. Synaptic conductance analysis methods based on the 
fluctuating point-conductance model

We presented how the simple point-conductance model of 
cortical synaptic activity can provide a basis for the analysis of 
experimental data, essentially through the matching of expres-
sions derived from the model to intracellular 
$V_m$ recordings of 
cortical neurons. This approach has been used for extracting 
different parameters from the recurrent cortical activity 
in vivo:
the averages and variances of excitatory and inhibitory conduc-
tances, their decay time constants and the optimal conductance 
waveform underlying spike selectivity. These analyses were 
possible because the point-conductance model represents in a 
compact and mathematically tractable way the activity resulting 
from several thousand synapses.

The $V_m$D analysis provides a characterization of synap-
thetic activity in simple terms (average conductance, level of 
fluctuations). Such parameters can readily be incorporated in 
computational models to yield the 
$V_m$ and conductance state 
corresponding to 
in vivo activity with just a few variables. This 
approach has been used for example in network simulations to 
obtain realistic conductance states in neurons even with small 
networks (Haeusler and Maass, 2007). It is also directly usable in 
dynamic-clamp experiments to investigate the impact of synap-
thetic background activity on signal processing by single cortical or 
thalamic neurons (Fellous et al., 2003; Shu et al., 2003; Wolfart 
et al., 2005; Desai and Walcott, 2006).

Beyond the matching of experimental 
$V_m$ distributions to a 
theoretical expression ($V_m$D method), we have also attempted 
to match the PSDs of 
$V_m$ fluctuations. This approach provides 
some validation for assumptions made about synaptic time con-
stants on the basis of published studies (Destexhe and Paré, 
1999; Destexhe et al., 2001). However, the fact that the point-
conductance model does not account for the scaling properties
Fig. 9. Spike-triggered conductance analysis in vivo. (A) STA conductance analysis from intracellular recordings in awake and sleeping cats. Two example cells are shown during wakefulness, and for each, the $V_m$ STA (top) and the extracted conductance STAs (bottom) are shown. In the first cell (left), the total conductance increases before the spike. In the second example cell (right), the total conductance decreases before the spike (black traces are exponential fits to the extracted STAs).

(B) Total conductance change preceding spikes as a function of the ratio $\sigma_e/\sigma_i$. Given the cell-to-cell variability of observed spike thresholds, each cell has a different predicted ratio separating total conductance increase cases from total conductance decrease cases. The two dashed lines ($\sigma_e/\sigma_i = 0.48$ and $\sigma_e/\sigma_i = 1.07$) visualize the two extreme predicted ratios. Cells in white are the ones not conforming to the prediction.

(C) Dependency of the ratio $\sigma_e/\sigma_i$ estimated by the $V_mD$ method on the value of the membrane capacitance $C$. Two sets of realistic $V_m$ distribution parameters were used as input for the estimation, one leading to $\sigma_e/\sigma_i > 0.6$ (left), another leading to $\sigma_e/\sigma_i < 0.6$ (right). For each set, the total input resistance was varied from 10 M$\Omega$ (bottom curves) to 50 M$\Omega$ (top curves), in steps of 10 M$\Omega$.

Panel A modified from Rudolph et al. (2007).

Finally, in order to be able to study average spike-triggered patterns of conductances in vivo, we have recently developed a probabilistic method for extracting the STAs of conductances from STAs of the $V_m$ (Pospischil et al., 2007). This method relies on the estimation of a number of parameters inherent to the neuron (like leak conductance and capacitance), as well as on synaptic conductance parameters estimated, for example, with the $V_mD$ method. We provided new data from dynamic-clamp experiments in vitro, demonstrating that given the synaptic conductance parameters, a good match between the extracted conductance STAs and the actual, known conductance STAs can be obtained.
4.2. Comparison to other synaptic conductance analysis methods

Other studies proposed synaptic conductance estimates derived from STA analysis: several-second-long current-clamp recordings of the membrane potential (in voltage-clamp) obtained at different points in time followed the same conductances: in this case, since the conductances ($G_L, g(t), g(t)$) are measured and the same leak conductance assumption), unless spikes are known and reproducible (for example, at a given delay after a sensory stimulation), so that the estimated, stimulus-locked conductance dynamics can be reasonably expected to be compatible with the voltage clamp due to high series resistance of patch electrodes.

The VmD method suffers from one common limitation with other approaches for synaptic conductance estimation: the need for discontinuous voltage-clamp with sharp electrodes (Haider et al., 2006). The fact that it relies on a forward reference that can be used. As mentioned above, all synaptic activity with TTX, but that also means recording a few points can be stressed. The fact that the VmD method can be compared to the analysis performed in the reviewed studies. However, this assumption also makes it unsuited, in its present form, for the analysis of stimulus-evoked activity with important temporal structure. In this relatively constraining experimental situation. The compromise chosen in the reviewed studies is with reference to the pre-stimulus activity, so that the estimates of this sign from dynamic-clamp experiments when a wrong leak conductance assumption made about the leak conductance, which excludes capacitance values tens of ms before the spike. The same effect is seen in vivo – V curves (in current-clamp) or V-I curves (in voltage-clamp) obtained at different levels of steady injected current.

4.3. Dependency of the STA extraction method on the VmD method

The result of the STA analysis is, however, dependent on the independence of excitation and inhibition allows the analysis methods to separate synaptic currents from leak currents. The fact that other approaches for synaptic conductance estimation: the need for discontinuous voltage-clamp with sharp electrodes (Haider et al., 2006). The fact that it relies on a forward reference that can be used. As mentioned above, all synaptic activity with TTX, but that also means recording a few points can be stressed. The fact that the VmD method can be compared to the analysis performed in the reviewed studies. However, this assumption also makes it unsuited, in its present form, for the analysis of stimulus-evoked activity with important temporal structure. In this relatively constraining experimental situation. The compromise chosen in the reviewed studies is with reference to the pre-stimulus activity, so that the estimates of this sign from dynamic-clamp experiments when a wrong leak conductance assumption made about the leak conductance, which excludes capacitance values tens of ms before the spike. The same effect is seen in vivo – V curves (in current-clamp) or V-I curves (in voltage-clamp) obtained at different levels of steady injected current.

4.4. Dynamic-clamp as a tool to evaluate conductance analysis methods

As to our probabilistic method for extracting the STAs of leak parameters of cortical cells any future experimental data providing information about the robustness of the estimates to the assumed leak conductance referenced analysis (as in Haider et al., 2006) could perhaps in a similar preparation (Pare et al., 1998) and checking the simple, classical protocol required for a subsequent VmD analysis.
4.5. Patterns of excitation and inhibition triggering spikes depend on the variances of the synaptic inputs.

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