Calculating Event-Triggered Average Synaptic Conductances From the Membrane Potential

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Pospischil M, Piwkowska Z, Rudolph M, Bal T, Destexhe A. Calculating event-triggered average synaptic conductances from the membrane potential. J Neurophysiol 97: 2544–2552, 2007. First published December 6, 2006; doi:10.1152/jn.01000.2006. The optimal patterns of synaptic conductances for spike generation in central neurons is a subject of considerable interest. Ideally such conductance time courses should be extracted from membrane potential (V_m) activity, but this is difficult because the nonlinear contribution of conductances to the V_m renders their estimation from the membrane equation extremely sensitive. We outline here a solution to this problem based on a discretization of the time axis. This procedure can extract the time course of excitatory and inhibitory conductances solely from the analysis of V_m activity. We test this method by calculating spike-triggered averages of synaptic conductances using numerical simulations of the integrate-and-fire model subject to colored conductance noise. The procedure was also tested successfully in biological cortical neurons using conductance noise injected with dynamic clamp. This method should allow the extraction of synaptic conductances from V_m recordings in vivo.

**INTRODUCTION**

Determining the optimal features of stimuli that are needed to obtain a given response is of considerable interest, for example, in sensory physiology. Reverse correlation is one of the most-used methods to obtain such estimates (Aguera y Arcas and Fairhall 2003; Badel et al. 2006) and, in particular, the spike-triggered average (STA) is often used to determine optimal features linked to the genesis of action potentials (de Boer and Kuypers 1968). The STA can be used to explore which feature of stimulus space the neuron is sensitive to or to identify modes that contribute either to spiking or to the period of silence before the spike (Aguera y Arcas and Fairhall 2003). Using intracellular recordings, it is straightforward to calculate the STA of the membrane potential (V_m), which yields the mean voltage trajectory preceding spiking. In contrast, it is much harder to determine the underlying synaptic conductance. Straightforward methods like recording at several different DC levels and estimating the total conductance from the ratio ΔV/Δt fail because the presence of a voltage threshold necessitates ΔV → 0 at the time of the spike, which, in turn, artificially suggests a divergence of the total conductance to infinity. Similarly, solving the membrane equation for excitatory and inhibitory conductances separately suffers from an additional complication: because the distance to threshold changes, the time courses of the average synaptic conductances depend on the injected current.

Recent contributions (Badel et al. 2006; Paninski 2006a,b) gave analytical expressions for the most likely voltage path, which in the low-noise limit approximates the STA of the leaky integrate-and-fire (IF) neuron. In those cases, Gaussian white noise current was considered as input. In Badel et al. (2006), a second state variable was added to obtain biophysically more realistic behavior. In Paninski (2006a,b), in addition the exact voltage STA for the nonleaky IF neuron was computed as well as the STA input current in discrete time. Here, a strong dependence of the STA shape on the time resolution dt was found without a stable limit as dt → 0. It was argued heuristically that this behavior results from the fact that decreasing the time step corresponds to increasing the bandwidth of the input current, a point which was supported by numerical simulations (Paninski et al. 2004; Pillow and Simoncelli 2003), in which a prefiltering of the white noise input results in a stable limit STA.

In this article, we focus on the problem of estimating the optimal conductance patterns required for spike initiation based solely on the analysis of V_m activity. We consider neurons subject to conductance based synaptic noise at both excitatory and inhibitory synapses. By discretizing the time axis, it is possible to obtain the probability distribution of conductance time courses that are compatible with the observed voltage STA. Due to the symmetry properties of the probability distribution, the STA time course of excitatory and inhibitory conductances can then be extracted by choosing the one with maximum likelihood. We test this method in numerical simulations of the IF model, as well as in real cortical neurons using the dynamic-clamp technique, by comparing the estimated STA with the real STA deduced from the injected conductances.

**METHODS**

**Models**

We considered neurons driven by synaptic noise described by two independent sources of colored conductance noise (point-conductance model (Destexhe et al. 2001)). The membrane equation of this system is given by

\[
C \frac{dV(t)}{dt} = -g_L(V(t) - V_L) - g_e(V(t) - V_e) - g_i(V(t) - V_i) + I_{DC}
\]

(1)

\[
g_e(t) = \frac{1}{\tau_e} \left( g_e(t) - g_e(t) \right) + \sqrt{2\sigma_e^2/\tau_e} \xi(t)
\]

(2)

Here, \(g_L, g_e(t),\) and \(g_i(t)\) are the conductances of leak, excitatory, and inhibitory currents; \(V_L, V_e, V_i\) are their respective reversal potentials, \(C\) is the capacitance and \(I_{DC}\) a constant current. The subscript \(s\) in Eq.

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In vitro experiments

In vitro experiments were performed on 0.4-mm-thick coronal or sagittal slices from the lateral portions of guinea-pig occipital cortex. Guinea pigs, 4–12 wk old (CPA, Olivet, France), were anesthetized by sodium pentobarbital (30 mg/kg). The slices were maintained in slice solution containing (in mM) 124 NaCl, 2.5 KCl, 1.2 MgSO4, 1.25 NaHPO4, 2 CaCl2, 26 NaHCO3, and 10 dextrose and aerated with 95% O2-5% CO2 to a final pH of 7.4. Intracellular recordings were made with a glass (WPI, 1BF100) and beveled on a Sutter Instruments beveler. The glass was drawn out on a Sutter Instruments P-87 micropipette puller from medium-walled glass. Bursts were recorded by glass recording electrodes with diameters of 1–5 μm and had resistances of 80–100 MΩ after beveling.

The dynamic-clamp technique (Robinson and Kawai 1993; Sharp et al. 1993) was used to inject computer-generated conductances in real neurons. Dynamic-clamp experiments were run using the hybrid RT-NEURON environment (developed by G. Le Masson, Université de Bordeaux), which is a modified version of NEURON (Hines and Carnevale 1997) running under the Windows 2000 operating system (Microsoft). NEURON was augmented with the capacity of simulating neuronal models in real time, synchronized with the intracellular recording. To achieve real-time simulations as well as data transfer to the PC for further analysis, we used a PCI DSP board (Innovative Integration) with four analog/digital (inputs) and four digital/analog (outputs) 16-bit converters. The DSP board constrains calculations of the models and data transfers to be made with a high priority level by the PC processor. The DSP board allows input (for instance the membrane potential of the real cell incorporated in the equations of the models) and output signals (the synaptic current to be injected into the cell) to be processed at regular intervals (time resolution = 0.1 ms). A custom interface was used to connect the digital and analog inputs/outputs signals of the DSP board with the intracellular amplifier (Axoclamp 2B, Axon Instruments) and the data-acquisition systems (PC-based acquisition software ELPHY, developed by G. Sadoc, CNRS Gif-sur-Yvette, ANVAR and Biologic). The dynamic-clamp protocol was used to insert the fluctuating conductances underlying synaptic noise in cortical neurons using the point-conductance model, similar to a previous study (Destexhe et al. 2001). According to Eq. 1, the injected current is determined from the fluctuating conductances $g_1(t)$ and $g_2(t)$ as well as from the difference of the membrane voltage from the respective reversal potentials, $I_{DnClamp} = -g_e(V - V_e) -g_i(V - V_i)$.

All research procedures concerning the experimental animals and their care adhered to the American Physiological Society’s Guiding Principles in the Care and Use of Animals, to the European Council Directive 86/609/EEC and to European Treaties Series 123 and was also approved by the local ethics committee “Ile-de-France Sud” (Certificate 05-003).

RESULTS

We first explain the method for extracting STAs from $V_m$ activity, then we present tests of this method using numerical simulations and intracellular recordings in dynamic clamp.

Method to extract conductance STA

The procedure we follow here to estimate STA of conductances from $V_m$ activity is based on a discretization of the time axis. With this approach, a probability distribution can be constructed for the maximum which gives the most likely conductance path compatible with the STA of the $V_m$. This maximum is determined by a system of linear equations that is solvable if the means and variances of conductances are known (for a method to estimate conductance mean and variance, see Rudolph et al. 2004).

We start from the voltage STA, which is an average over an ensemble of event-triggered voltage traces. Its relation to the conductance STAs is determined by the ensemble average of Eqs. 1 and 2. In general, there is a strong correlation (or anti-correlation) between $V(t)$ and $g(t)$ in time. However, it is safe to assume that there is no such correlation across the ensemble, since the noise processes $ξ(t)$ corresponding to each realization are uncorrelated. Also, the ensemble average is commutative with the time derivative. Thus we can rewrite Eqs. 1 and 2 to obtain

$$\frac{d(V(t))}{dt} = \frac{1}{\tau} ((V(t))_s - V(t)) - \frac{\langle \xi(t)_s \rangle}{C} ((V(t))_s - V(t))$$

$$\frac{d(g(t))}{dt} = \frac{1}{\tau} ((g(t))_s - g(t)) + \frac{2\sigma^2_\xi}{\tau} \langle \xi(t)_s \rangle,$$
We discretize Eq. 3 in time with a step-size $\Delta t$ and solve for $g_i^k$:

$$g_i^k = -\frac{C}{V^i - V_e} \left\{ \frac{V^i - V_e}{\tau_i} + \frac{g_i^k(V^i - V_e)}{C} + \frac{V^{i+1} - V^i}{\Delta t} - \frac{I_{\text{ele}}}{} \right\}$$  \hspace{1cm} (5)

Because the series $V^i$ for the voltage STA is known, $g_i^k$ has become a function of $g_e^k$. In the same way, we solve Eq. 4 for $g_e^k$, which have become Gaussian-distributed random numbers

$$g_e^k = \frac{1}{\sigma_e} \sqrt{\frac{\tau_e}{2\Delta t}} \left( g_e^{k+1} - g_e^k \right) \left( 1 - \frac{\Delta t}{\tau_e} \right) - \frac{\Delta t}{\tau_e} g_{\text{ele}}$$  \hspace{1cm} (6)

There is a continuum of combinations \{g^{k+1}_e, g^k_e\} that can advance the membrane potential from $V^{k+1}$ to $V^{k+2}$, each pair occurring with a probability

$$p^k := p(g^{k+1}_e, g^k_e | g^{k+1}_e, g^k_e) = \frac{1}{2\pi} e^{-\frac{(x^k - \mu_k)^2}{2\sigma_k^2}}$$  \hspace{1cm} (7)

The effect of negative conductances becomes dominant. In this example, where the ratio SD/mean was fixed at 0.1, the RMS deviation enters a plateau at $7,000$ spikes. The plateau values can also be recovered from the neighboring plots (i.e., the RMS deviations at SD/mean = 0.1 in Fig. 2A shows the RMS deviation per spike). On the other hand, a broadening of the conductance distribution yields a higher deviation between simulation and estimation. However, at SD/mean = 0.5, the RMS deviation is still as low as $\sim 2\%$ of the mean conductance for excitation and $\sim 4\%$ for inhibition.

To test the effect of dendritic filtering on the reliability of the method, we used a two-compartment model based on that of Pinsky and Rinzel (1994), from which we removed all active channels and replaced them by an integrate-and-fire mechanism at the soma. We repeatedly injected the same 100-s sample of fluctuating excitatory and inhibitory conductances in the dendritic compartment and performed two different recording protocols at the soma (Fig. 3A). First, we recorded in current clamp to obtain the $V_m$ time course as well as the spike times. In this case, the leak conductance $g_0^{\text{leak}}$ and the capacitance $C^{\text{m}}$ were obtained from current pulse injection at rest. Second, we simulated an “ideal” voltage clamp (no series resistance) at the soma using two different holding potentials (we chose the reversal potentials of excitation and inhibition, respectively). Then, from the currents $I_e$ and $I_i$, one can calculate the conductance time courses as

$$g_e^k(t) = \frac{I_e(t) - g_e(V_m - V_e)}{V_i - V_e}$$  \hspace{1cm} (11)

where the superscript $s$ indicates that these are the conductances seen at the soma (in the following referred to as somatic conductances). From these, we determined the parameters $g_k^{\text{leak}}$, $g_i^{\text{leak}}$, $\sigma_k^{\text{leak}}$, and $\sigma_i^{\text{leak}}$, the conductance means and SDs. In contrast to $g_e(t)$ and $g_i(t)$, the distributions of $g_k^{\text{leak}}(t)$ and $g_i^{\text{leak}}(t)$ are not Gaussian (not shown), and they have mean lower and variances. We compared the STA of the injected (dendritic) conductance, the STA obtained from the somatic $V_m$ using our method and the STA obtained using a somatic “ideal” voltage clamp (see Fig. 3B–D), which demonstrated the following points: as expected, due to dendritic attenuation, all somatic estimates were saturated compared with the actual conductances in-
FIG. 1. Test of the spike-triggered average (STA) analysis method using an IF neuron model subject to colored conductance noise. A: scheme of the procedure used. An integrate-and-fire (IF) model with synaptic noise was simulated numerically (bottom) and the procedure to estimate STA was applied to the voltage $V_m$ activity (top). The estimated conductance STAs from $V_m$ were then compared with the actual conductance STAs in this model. Bottom: STA analysis for different conditions, low-conductance states (B and C), high-conductance states (D and E) with fluctuations dominated by inhibition (B and D) or by excitation (C and E). For each panel, the top graph shows the voltage STA, the middle graph the STA of excitatory conductance, and the bottom graph the STA of inhibitory conductance. Solid lines (gray) show the average conductance recorded from the simulation, whereas the dashed line (black) represents the conductance estimated from the $V_m$. Parameters in B: $g_e = 20 \text{nS, } g_i = 60 \text{nS, } \sigma_e = 1.5 \text{nS, } \sigma_i = 1.5 \text{nS;}$ C: $g_e = 20 \text{nS, } g_i = 60 \text{nS, } \sigma_e = 4 \text{nS, } \sigma_i = 12 \text{nS;}$ D: $g_e = 20 \text{nS, } g_i = 60 \text{nS, } \sigma_e = 6 \text{nS, } \sigma_i = 3 \text{nS.}$

FIG. 2. The root-mean-square (RMS) of the deviation of the estimated from the recorded STAs. A: RMS deviation as a function of the number of spikes for the STA of excitatory conductance where the SD of the conductance distribution was 10% of its mean. The RMS deviation first decreases with the number of spikes but saturates at $\sim 7,000$ spikes. This is due to the effect of negative conductances, which are excluded in the simulation (cf. C). B: same as A for inhibition. C: RMS deviation for excitation as a function of the ratio SD/mean of the conductance distribution. The higher the probability of negative conductances, the higher the discrepancy between theory and simulation. However, at SD/mean = 0.5, the mean deviation is as low as $\sim 2\%$ of the mean conductance for excitation and $\sim 4\%$ for inhibition. D: same as C for inhibition.
jected in dendrites (compare light and dark gray curves, soma, with black curve, dendrite, in Fig. 3, B–D); the estimate obtained by applying the present method to the somatic \( V_m \) (dark gray curves in Fig. 3, B–D) was very similar to that obtained using an “ideal” voltage-clamp at the soma (light gray curves). The difference close to the spike may be due to the non-Gaussian shape of the somatic conductance distributions, the tails of which then become important; despite attenuation, the qualitative shape of the conductance STA was preserved. We conclude that the STA estimate from \( V_m \) activity captures rather well the conductances as seen by the spiking mechanism.

**Test of the method in real neurons**

We also tested the method on voltage STAs obtained from dynamic-clamp recordings of guinea pig cortical neurons in slices. In real neurons, a problem is the strong influence of spike-related voltage-dependent (presumably sodium) conductances on the voltage time course. Because we maximize the global probability of \( g_e(t) \) and \( g_i(t) \), the voltage in the vicinity of the spike has an influence on the estimated conductances at all times. As a consequence, without removing the effect of sodium, the estimation fails (see Fig. 4). Fortunately, it is rather simple to correct for this effect by excluding the last 1–2 ms

![Diagram](image-url)
before the spike from the analysis. The corrected comparison between the recorded and the estimated conductance traces is shown in Fig. 5.

Finally, to check for the applicability of this method to in vivo recordings, we assessed the sensitivity of the estimates with respect to the different parameters by varying the values describing passive properties and synaptic activity. We assume that the total conductance can be constrained by input resistance measurements and that time constants of the synaptic currents can be estimated by power spectral analyses (Destexhe and Rudolph 2004). This leaves \( g_L, C, g_e, \sigma_e, \sigma_i \) and \( C \) as the main parameters. The impact of these parameters on STA conductance estimates is shown in Fig. 6. Varying these parameters within \( \pm 50\% \) of their nominal value led to various degrees of error in the STA estimates. The dominant effect of a variation in the mean conductances is a shift in the estimated STAs, whereas a variation in the SDs changes the curvature just before the spike.

To address this point further, we fitted the estimated conductance STAs with an exponential function

\[
f_s(t) = G_s(1 + K_e \exp^{-t/t_e}),
\]

where \( s \) again takes the values \( e, i \) for excitation and inhibition, respectively. \( t_0 \) is chosen to be the time at which the analysis stops. Figure 7 gives an overview of the dependence of the fitting parameters \( G_e, G_i, T_e, T_i \) on the relative change of \( g_L, g_{e0}, \sigma_e, \sigma_i \), and \( C \). For example, a variation of \( g_{e0} \) has a strong effect on \( G_e \) and \( G_i \) but affects to a lesser extent \( T_e \) and \( T_i \), whereas the opposite was seen when varying \( \sigma_e \) and \( \sigma_i \).

**DISCUSSION**

Understanding the transfer function of a neuron from synaptic input to spike output would ideally require the simultaneous monitoring of both the synaptic conductances and the cell’s firing. Current methods for extracting synaptic conductances rely on intracellular recordings performed at different holding potentials (in voltage clamp) or different current levels (in current clamp) (e.g., Borg-Graham et al. 1998), and, as a consequence, they do not allow the establishment of a direct correspondence between synaptic conductances and spikes.
Although these methods have been very useful, for example in establishing the synaptic structure of sensory receptive fields in a variety of systems (Monier et al. 2003; Wehr and Zador 2003; Wilent and Contreras 2005), they do not distinguish between trials that effectively produce spikes at a given latency and those that do not.

Here we have presented a method to extract the average excitatory and inhibitory conductance patterns directly related to spike initiation. As illustrated in Fig. 8, this method can extract spike-related conductances based solely on the knowledge of $V_m$ activity. First, the STA of the $V_m$ is computed from the intracellular recordings. Next, by discretizing the time axis, one estimates the “most likely” conductance time courses that are compatible with the observed STA of $V_m$. Due to the symmetry of their distribution, the average conductance time courses coincide with the most likely ones so integration over the entire stimulus space (the dimension of which depends on the STA interval as well as on the temporal resolution) can be replaced by a differentiation and subsequent solution of a system of linear equations. Solving this system gives an esti-

**FIG. 6.** Deviation in the estimated conductance STAs in real neurons using dynamic clamp due to variations in the parameters. The black lines represent the conductance STA estimates using the correct parameters, the gray areas are bound by the estimates that result from variation of a single parameter (indicated on the right) by $\pm 50\%$. Light gray areas represent inhibition, dark gray areas represent excitation. The total conductance (leak plus synaptic conductances) was assumed to be fixed. A variation in the mean values of the conductances evokes mostly a shift in the estimate, whereas a variation in the SDs influences the curvature just before the spike.

**FIG. 7.** Detailed evaluation of the sensitivity to parameters. The conductance STAs were fitted with an exponential function $f_s(t) = G_s[1 + K_s \exp((t - t_0)/T_s)]$, $s = e, i$, $t_0$ is chosen to be the time at which the analysis stops. Each plot shows the estimated value of $G_e, G_i, T_e, T_i$ from this experiment, each curve represents the variation of a single parameter (see legend).
should be complemented by a check for I-V curve linearity in the range of $V_m$ considered. Note that a linear I-V curve does not guarantee the absence of voltage-dependent conductances. For example, if the mean interspike interval of the cell becomes too short, spike-related potassium currents might be present during a substantial fraction of the STA interval and could affect the estimation. This might diminish the applicability of the method to neurons spiking at high frequency, in particular to fast-spiking interneurons. Also, strong subthreshold dendritic conductances that are very remote from the soma could influence the STA estimate without being visible in the I-V curve. On the other hand, in cases where it is possible to parameterize these nonlinearities, they can be included in Eq. 5. It should thus be possible to extend the method to apply it to more complex models, for example the exponential IF model (Fourcaud-Trocme et al. 2003). Another possible extension would be to include voltage-dependent terms such as N-methyl-D-aspartate (NMDA) receptor-mediated synaptic currents, although such currents probably have a limited contribution at the range of $V_m$ considered here (below $-50$ mV).

Another source of error may arise from “negative conductances.” The present model of synaptic noise assumes that conductances are Gaussian-distributed, but if the SD becomes comparable to the mean value of the conductances, the Gaussian distribution will include negative conductances, which are unrealistic. This is an important limitation of representing synaptic conductances by Gaussian-distributed noise ("diffusion approximation"). However, this type of approximation seems to apply well to cortical neurons in vivo, which receive a large number of inputs (Destexhe et al. 2001). In vivo measurements so far indicate that the SD is much smaller than the mean for both excitatory and inhibitory conductances (Haider et al. 2006; Rudolph et al. 2005), which also indicates that the diffusion approximation is valid in this case. Such a check for consistency is a prerequisite for applying the present method.

Previous work related to the question of spike-triggered stimuli was mainly focused on white noise current inputs and showed that no stable finite input average exists in the limit $dt \to 0$ (Paninski 2006a). Other contributions shed light on the question of membrane voltage STAs for the leaky IF neuron as well as for biophysically more plausible models. However, so far no procedure was proposed to solve this problem of reverse correlation for conductance noise inputs. The method we propose here attempts to fill this gap and directly provides a procedure that can be applied to real neurons. To this end, the present method must be complemented by measurements of the mean and SD of excitatory and inhibitory conductances. Such measurements can be obtained either by voltage clamp (Haider et al. 2006), or by current clamp as recently proposed (Rudolph et al. 2004, 2005). Combining the latter method with the present method, it should now be possible to directly extract conductance patterns from $V_m$ recordings in vivo and thus obtain estimates of the conductance variations related to spikes during natural network states.

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