High-resolution intracellular recordings using a real-time interaction between the neuron and a computational model of the electrode
Supplementary Methods

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Contents

1 Biological preparation

We prepared 380 μm-thick coronal or sagittal slices from the lateral portions of 4-12 week old guinea-pig (CPA, Olivet, France) occipital cortex, as well as from adult ferret (Marshall, France) occipital cortex in some early experiments, as described previously [1, 2]. 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 $MgSO_4$, 1.25 $NaHPO_4$, 2 $CaCl_2$, 26 $NaHCO_3$, and 10 or 25 dextrose, and aerated

with 95% O_2-5% CO_2 to a final pH of 7.4. Intracellular recordings were performed in all cortical layers after 2h of recovery. 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).

2 Electrophysiology

Sharp electrodes for intracellular recordings were made on a Sutter Instruments P-87 micropipette puller from medium-walled glass (WPI, 1BF100) and bevelled on a Sutter Instruments beveller (BV-10M). Micropipettes were filled with 1.2-2 M potassium acetate - 4 mM potassium chloride and had resistances of 65-110 MΩ after bevelling. An Axoclamp 2B amplifier (Axon Instruments) was used for V_m recording and current injection. A Digidata 1322A card (Axon Instruments) and the PC-based software ELPHY (developed by G. Sadoc, CNRS Gif-sur-Yvette, ANVAR and Biologic) were used for data acquisition at 20 kHz. The Axoclamp was used either in continuous current-clamp ('bridge') mode or in discontinuous current-clamp (DCC) mode. In both cases, the capacitance neutralization was set at the maximal possible value, slightly before the onset of oscillations, to achieve the fastest possible electrode charging time (as assessed by the Monitor output viewed at high temporal resolution on an analogical oscilloscope, allowing to adjust the time constant of the electrode response decay after injection of ultra-short current pulses in discontinuous mode). The switching frequency of the DCC mode was chosen accordingly, so that the sampling of the V_m took place after the electrode transient had decayed to a plateau (`http://www.moleculardevices.com/pages/instruments/axon_guide.html`).

3 Real-time computer implementation

We used the hybrid RT-NEURON environment (developed by G. Le Masson, INSERM 358, Université Bordeaux 2), a modified version of NEURON [3] running under the Windows 2000 operating system (Microsoft). To achieve real-time electrode compensation and simulation of synaptic inputs (dynamic-clamp) 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 made by NEURON and data transfers to be made with a high priority level by the PC processor. The DSP board allows input (the total recorded potential $V_m + U_e$ to be compensated, and then incorporated in the equations of the models in the dynamic-clamp case) and output signals (the current to be injected into the cell; the compensated V_m for the acquisition system) to be processed at regular intervals (0.1 ms time resolution). A

custom interface or a CyberAmp 380 (Axon Instruments) were used to low-pass-filter at 6 kHz the analog input/output signals of the DSP board and to adjust their ranges in order to improve the digitalization resolution.

4 Data analysis

The PC-based software ELPHY (developed by G. Sadoc, CNRS Gif-sur-Yvette, ANVAR and Biologic), Matlab (The Mathworks, Natick, MA) , Scilab (INRIA / ENPC, `http://www.scilab.org`) and custom-written C-code were used. All statistical tests were performed using the software Statview 5.0 (SAS Institute, Cary, N.C.). All values are given as average $\pm$ standard deviation or, for small sample size, as average and range. A P-value < 0.05 was required for statistical significance.

4.1 White noise injection

Since the injected subthreshold current noise had a mean of 0 nA, we only compared the standard deviations of recorded V_m distributions with theoretical values of standard deviations predicted from the noise parameters and passive neuron parameters derived from responses to small current pulses. Relative error is:

$$100 \times \frac{|\sigma_{\text{experiment}} - \sigma_{\text{theory}}|}{\sigma_{\text{theory}}}$$

and

$$\sigma_{\text{theory}} = R\sqrt{\frac{dt}{2\tau_m}}\sigma_I$$

where R is the input resistance, τ_m is the membrane time constant, dt is time step (0.1 ms in our experiments) and σ_I is the standard deviation of the input current (for a single sampling step).

4.2 Square conductance pulses

For each train of injected pulses, an initial stable part of the response was selected for analysis (as assessed by a non-significant Spearman non-parametric test for correlation between response amplitude and time), in order to minimize deviations from the theoretical prediction due to small drifts in the recording.

Recordings were analyzed on phase plots of $V_m(t + T/4)$ vs. $V_m(t)$ (where T is the period of the waveform), where predicted responses for a passive membrane are squares. We fit an optimal square to the experimental phase plot (least square minimization of the average distance of experimental points to the square), then compared the length of the side and the tilt between the optimal square and the theoretical square (equations below). The level of noise in the experimental trace was quantified by the average distance (in mV) of the data points to the optimal square.

In each cell, a given parameter configuration is always tested in both AEC and DCC. Differences between the two conditions are thus tested with a paired statistical test, the Wilcoxon non-parametric test. Correlations between measures derived from the phase plots and stimulus parameters were evaluated using simple linear regression analysis: the P-values given correspond to the null hypothesis that the slope of the linear regression is 0 (two-tailed t-test). For descriptive statistics and linear regression analyses, one data point was excluded (for both methods and all error measures): given the very small theoretical prediction for the side measure, in one case of 1000 Hz stimulus frequency, the relative error on the side measure was extremely large (due to division by this very small value) and constituted a clear outlier. The removal of this one point did not affect the results of the Wilcoxon tests nor of most linear regression analyses.

Response of a passive membrane to square conductance pulses

We consider a passive membrane model with a square conductance wave stimulation described by the following equation:

$$C\frac{dV_m}{dt} = -g_L(V_m - V_{\text{rest}}) - g^+(t)(V_m - E^+) - g^-(t)(V_m - E^-)$$

where C is the membrane capacitance, g_L is the leak conductance ($1/R$), E^+ and E^- are reversal potentials, and $g^+(t)$ and $g^-(t)$ are time-varying conductances. The conductances are alternating square pulses, i.e., $g^+(t) = g$ and $g^-(t) = 0$ when $t \bmod T \in [0, T/2[$, and $g^-(t) = g$ and $g^+(t) = 0$ when $t \bmod T \in [T/2, T[$, where T is the period and g is the maximum conductance.

Because the total conductance $g + g_L$ is constant, the response $V_m(t)$ is piecewise exponential with time constant $(g + g_L)/C$. After the response settles in the stationary regime, it is periodic (period of the stimulus T) and consists of an alternation of two pieces of exponential functions, of the form $a+b\exp(-(g+g_L)t/C)$. It follows for any given delay d, $V(t + d)$ and $V(t)$ are related by an affine relation on every smooth piece, and there are $2 \times 2 = 4$ different sets of parameters. Therefore the phase plot defined by the graph of $(V(t), V(t+d))$ is a quadrilateral. When $d = T/4$, symmetry arguments imply that it must be square.

The corners of the square correspond to the discontinuity points of the conductance pulses. Solving the differential equation gives an explicit formula for their coordinates. The coordinates of the center of the square are

$$(C_x, C_y) = (\frac{g}{g + g_L}\frac{E^+E^-}{2}, \frac{g}{g + g_L}\frac{E^+E^-}{2})$$

and the coordinates of the first corner are

$$(M_x, M_y) = (C_x + \frac{A}{2}, C_y + \frac{A}{2(1 + \alpha)})$$

where

$$A = \frac{g}{g+g_L}(E^+ - E^-)\frac{1-\alpha^2}{1+\alpha^2}$$
$$\alpha = \exp(-\frac{(g+g_L)T}{4C})$$

The three other corners are obtained by rotations around the center C with angles of 90°, 180° and 270°.

4.3 Colored conductance noise

Fragments of responses to fluctuating conductance injection (see [4] for stochastic equations generating these conductances; parameters used here were similar) were selected so as to avoid rare spikes or rare, very sharp electronic artefacts (seen in some recordings independent of compensation method or protocol used) which introduce spurious high frequencies. V_m distributions were obtained for each fragment and their parameters were averaged over fragments. Theoretical V_m distributions were computed using a steady-state solution of the passive membrane equation (see [1] for equations) and known injected conductance and passive membrane parameters (derived from response to small current pulses). The difference between theory and experiment was quantified by the relative error:

$$100 \times \frac{|\text{param}_{\text{experiment}} - \text{param}_{\text{theory}}|}{\text{param}_{\text{theory}}}.$$

The Wilcoxon test was used to assess whether the relative errors for AEC and for DCC were significantly different. Each fragment was also fit by the theoretical template of the PSD [5] using a simplex fitting algorithm [6]. The theoretical template was:

$$S_V(\omega) = \frac{1}{1+\omega^2\,\tilde{\tau}_m^2}\left[\frac{A_e\,\tau_e}{1+\omega^2\,\tau_e^2} + \frac{A_i\,\tau_i}{1+\omega^2\,\tau_i^2}\right].$$

where $\omega = 2\pi f$, f is the frequency, $\tilde{\tau}_m$ is the effective membrane time constant, A_e and A_i are amplitude parameters and τ_e and τ_i time constants for excitation and inhibition, respectively. Fits were realized by fitting both A_e and A_i, as well as τ_e and τ_i. $\tilde{\tau}_m$ was fixed to the value estimated from the recordings. Different initial conditions ("first guesses") were given to the fitting procedure to ensure that there was no convergence to local minima. Fit errors were averaged over fragments (Fig. 5f).

4.4 Spike onset analysis

Spikes were triggered by a realistic dynamic-clamp 300s-long protocol combining randomized injection of AMPA synaptic inputs of 5 different amplitudes at 10 Hz

and injection of a background of colored conductance noise [4] in either a "high conductance" ($g_i > g_e$), or a "low conductance" configuration ($g_e = g_i$) (Z.P. et al, `http://arxiv.org/abs/0706.1306`). For each injection protocol, an initial stable part of the response was selected for analysis (as assessed by a non-significant Spearman non-parametric test for correlation between spike threshold and time). 914 spikes were analyzed on average for each protocol (range 251-1895 spikes). Both AMPA-triggered and spontaneous spikes were analyzed together. Spike threshold was detected using a threshold on the derivative of the V_m (similar to procedures described in the literature, see [7]): for the analysis shown on fig 6, a threshold of 83.3 mV/ms is used for both AEC and DCC injections. The smoothed DCC trace was obtained by replacing each data point i by the average of 9 surrounding points (i.e. an average from $i-4$ to $i+4$). The 9-point size of the sliding window was chosen by visual inspection of the resulting trace in order to produce spikes that maximally resemble the spikes recorded in AEC. The slope of the depolarization preceding the spike is computed by a linear regression on 2.4 ms before the spike (excluding the data point at which the threshold is detected). Spike-triggered averages of theV_m show that the average depolarization preceding the spike has a duration around this value in these protocols (not shown). Both a linear and an exponential function were fit to the clouds of points representing the threshold of each spike as a function of the slope of the preceding depolarization. The exponential fits were in some cases slightly better than the linear ones, but in other cases they were indistinguishable: since in those cases the time constants of the exponential fits have aberrant values, the exponential fits were not analyzed further. The significance of the correlation between slope of depolarization and spike threshold was assessed using a non-parametric Spearman correlation test.

5 Kernel estimation

5.1 The least square problem

We assume that the neuron and the electrode respond linearly to the injected currents we use. We will discuss this hypothesis later. Then the recorded potential V in response to an input current I is the linear convolution:

$$V(t) = V_0 + (K * I)(t) = V_0 + \int_0^{+\infty} K(s)I(t-s)ds$$

where K is the impulse response of the system (neuron + electrode), which we call the *kernel*, and V_0 is the resting potential. In the digital domain, the formula reads

$$V_n = V_0 + \sum_0^{+\infty} K_p I_{n-p}$$

If the time-varying current I is known and V is measured over a long enough period of time, then it is possible to calculate the kernel K. Assuming that the

measure is corrupted by gaussian noise, the best estimation of K is the solution of the linear least square problem, i.e., ($\mathbf{K}$,V_0) minimizes

$$E = \sum_{n=0}^{N-1} \left(V_n - V_0 - \sum_{p=0}^{+\infty} K_p I_{n-p} \right)^2$$

where N is the number of measurements, i.e., $N\Delta$ is the duration of the stimulation, where Δ is the sampling step ($\Delta = 0.1$ ms in our experiments). Typically, the stimulation lasts 5 to 20 s, which corresponds to 50000 – 200000 measurements.

From $\frac{\partial E}{\partial V_0} = 0$ and $\frac{\partial E}{\partial K_i} = 0$ for all i we find:

$$\begin{aligned} \forall i \geq 0, \sum_{n=0}^{N-1} V_n I_{n-i} &= \sum_{p=0}^{+\infty} K_p \sum_{n=0}^{N-1} I_{n-p} I_{n-i} + V_0 \sum_{n=0}^{N-1} I_{n-i} \\ \sum_{n=0}^{N-1} V_n &= \sum_{p=0}^{+\infty} K_p \sum_{n=0}^{N-1} I_{n-p} + N V_0 \end{aligned}$$

with the convention $I_k = 0$ when $k < 0$ (no input current before time 0). In the following we define $\langle x_n \rangle = \frac{1}{N} \sum_{n=0}^{N-1} x_n$ (average over all samples).

In practice, we consider only the first M steps of the kernel K, so that the equations above can be expressed as a matrix problem $\mathbf{AX} = \mathbf{B}$, where $\mathbf{A}$ is a square matrix with coefficients $a_{i,j} = \langle I_{n-j} I_{n-i} \rangle$ for $i, j \in \{0 \dots M-1\}$, $a_{i,M} = \langle I_{n-i} \rangle$ for $i \in \{0 \dots M-1\}$, $a_{M,j} = \langle I_{n-j} \rangle$ for $j \in \{0 \dots M-1\}$ and $a_{M,M} = 1$; $\mathbf{X}$ is a column vector with $X_i = K_i$ for $i \in \{0 \dots M-1\}$ and $X_M = V_0$; $\mathbf{B}$ is a column vector with $B_i = \langle V_n I_{n-i} \rangle$ for $i \in \{0 \dots M-1\}$ and $B_M = \langle V_n \rangle$. Solving this linear equation for $\mathbf{X}$ gives the coefficients of the kernel K and the resting potential V_0.

5.2 Fast implementation

Although there is no theoretical problem in solving the linear problem described above, the matrix $\mathbf{A}$ can be large and each coefficient $a_{i,j}$ is a sum over all samples. But we note that in the limit $N \to +\infty$ (infinite number of samples) $\langle I_{n-i} I_{n-j} \rangle = \langle I_n I_{n+i-j} \rangle$ for a stationary current. In this case the matrix $\mathbf{A}$ has only $M+1$ distinct coefficients. However in practice the number of samples is finite, so that for $j > i$,

$$\begin{aligned} \langle I_{n-i} I_{n-j} \rangle &= \frac{1}{N} \sum_{n=-i}^{N-1-i} I_n I_{n+i-j} \\ &= \langle I_n I_{n+i-j} \rangle - \frac{1}{N} \sum_{n=N-i}^{N-1} I_n I_{n+i-j} \end{aligned}$$

In general, the correction term vanishes only when $N \to +\infty$, but we can ensure that it also vanishes for finite N by enforcing $I_n = 0$ for all $n \in \{N - M +$

$1 \dots N-1\}$, i.e., there is no input current at the end of the stimulation. In the same way, $\langle I_{n-i} \rangle = \langle I_n \rangle$ for all $i \in \{0 \dots M-1\}$.

Then we define $a_k = \langle I_n I_{n-k} \rangle$ for all $k \in \{0 \dots M-1\}$, and $y = \langle I_n \rangle$, so that the matrix $\mathbf{A}$ can be written as follows:

$$\mathbf{A} = \begin{pmatrix} a_0 & a_1 & a_2 & \dots & a_{M-1} & y \\ a_1 & a_0 & a_1 & \dots & a_{M-2} & y \\ a_2 & a_1 & a_0 & \dots & a_{M-3} & y \\ \dots & \dots & \dots & \dots & \dots & y \\ a_{M-1} & a_{M-2} & a_{M-3} & \dots & a_0 & y \\ y & y & y & \dots & y & 1 \end{pmatrix} = \begin{pmatrix} \tilde{\mathbf{A}} & \mathbf{Y} \\ \mathbf{Y}^T & 1 \end{pmatrix}$$

We also define $\mathbf{X} = \begin{pmatrix} \mathbf{K} \\ V_0 \end{pmatrix}$ and $\mathbf{B} = \begin{pmatrix} \tilde{\mathbf{B}} \\ \langle V_n \rangle \end{pmatrix}$ and $\mathbf{Y}^T = \begin{pmatrix} y & y & \dots & y \end{pmatrix}$. Solving the matrix equation by block gives

$$\begin{aligned} V_0 &= \langle V_n \rangle - \mathbf{Y}^T \mathbf{K} = \langle V_n \rangle - \langle I_n \rangle \sum_{p=0}^{M-1} K_p \\ (\tilde{\mathbf{A}} - \mathbf{Y}\mathbf{Y}^T)\mathbf{K} &= \tilde{\mathbf{B}} - \langle V_n \rangle \mathbf{Y} \end{aligned}$$

The coefficients of the matrix $\mathbf{U} = \tilde{\mathbf{A}} - \mathbf{Y}\mathbf{Y}^T$ are $u_{i,j} = a_{|i-j|} - \langle I_n \rangle^2$. The coefficients of the vector $\tilde{\mathbf{B}} - \langle V_n \rangle \mathbf{Y}$ are $\langle V_n I_{n-i} \rangle - \langle V_n \rangle \langle I_n \rangle$. The matrix $\mathbf{U}$ is a Toeplitz matrix, and solving a linear problem for a Toeplitz matrix can be done very quickly with the use of the Levinson-Durbin algorithm (which is documented for example in [6]).

It appears that it is not necessary to store all the values of V_n and I_n. The averages $\langle V_n I_{n-j} \rangle$ can be computed online in real time (M additions at each time step).

5.3 Choosing the input signal

The input signal I must chosen so that

1. it is zero at the end of the stimulation (last M steps, where M is the kernel size) so as to use the Levinson-Durbin algorithm, as shown in the previous section;
2. the neuron response is essentially linear;
3. it makes the best possible use of the D/A converters of the acquisition board.

Constraints 2 is satisfied by letting (I_n) be a sequence of independent random numbers with appropriate variance. Constraint 3 is satisfied by letting each current step I_n be a random number with uniform distribution in the range of D/A converter. Thus, the input current is a stationary non-gaussian white noise (digitally sampled). We discuss this choice in the following.

Linearity of the membrane response

In general, the membrane potential does not respond linearly to the input current. However, it can be considered as locally linear around a given value of the potential; our strategy is thus to inject a signal that has a small effect on the membrane and a large effect on the electrde.

Because the electrode time constant is much smaller than the membrane time constant, the choice of a white noise input signal ensures that the membrane potential will not vary much while the electrode potential will vary much more. Indeed, the standard deviation of the response of a membrane with time constant τ_m and resistance R_m to a white noise is proportional to $R_m/\sqrt{\tau_m}$. Thus, if the electrode has time constants τ_e and resistance R_e then the ratio of electrode response over membrane response is

$$\frac{R_e}{R_m}\sqrt{\frac{\tau_m}{\tau_e}}$$

For a sharp electrode, the electrode and membrane resistances have the same magnitude and with a properly adjusted recording setup, $\tau_m \approx 100\tau_e$, so that the electrode response is about 10 times larger than the membrane response. Thus it is possible to ensure that the membrane potential remains within about 1 mV of its resting potential while the recorded potential varies by 10 mV on average.

Besides, we remark that the linearity of the membrane response is not so crucial in the estimation procedure because in cases when the response is non-linear, the algorithm finds the best linear approximation (in the least square sense).

Determining the level of noise injection

To estimate the kernel K, we inject a noisy current consisting of a sequence of independent random current steps at sampling resolution Δ, with amplitude uniformly distributed between $-I_{\max}$ and $+I_{\max}$. $I_{\max}$ is chosen so that the membrane potential remains close to its resting level, while the electrode response is large enough so as to maximize the signal/noise ratio. For an ideal electrode (i.e., very fast compared to the membrane), the membrane response is piecewise exponential, it is a low-pass filtered version of $R_m I(t)$ with time constant τ_m, where R_m is the membrane resistance, $I(t)$ is the injected current and τ_m is the membrane time constant. The standard deviation σ_V of the membrane potential is then given by the following formula:

$$\sigma_V = \sqrt{\frac{1-e^{-\frac{\Delta}{\tau_m}}}{1-e^{-\frac{\Delta}{\tau_m}}}} R_m \sigma_I \approx \sqrt{\frac{\Delta}{6\tau_m}} R_m I_{\max}$$

where σ_I is the standard deviation of the injected current, and assuming that the sampling step Δ is small compared to the membrane time constant τ_m. With the values $\Delta = 0.1$ ms, $\tau_m = 10$ ms, $R_m = 40$ MΩ and $I_{\max} = 0.5$ nA, we obtain $\sigma_V = 0.8$ mV, which is small enough. The expression we derived applies to an

ideal electrode; for non-ideal electrodes (which filter the injected current), it gives an upper bound for σ_V (approximately, τ_m is replaced by $\tau_m + \tau_e$, where τ_e is the electrode time constant). The electrode time constant is of the same order as the sampling step, therefore the electrode response can occasionally be close to the upper bound $R_e I_{\max}$, where $R_e$is the electrode resistance. It is crucial to estimate the range of the measured signal in order to adjust the acquisition system correctly. With $R_e = 50$ MΩ and $I_{\max} = 0.5$ nA, the range is ± 25 mV, which was appropriate for our acquisition system.

5.4 Isolation of electrode kernel

Once the kernel of the system neuron+electrode has been determined, the electrode kernel remains to be extracted. The idea is that the membrane is much slower than the electrode, so that we can distinguish the two contributions in the full kernel.

As a first approximation, we can write $K = K_m + K_e$, where K_m is the membrane kernel and K_e is the electrode kernel. We suppose that, in the regime in which the kernel was obtained (i.e., small white noise injection), the membrane responds approximately as a first order low-pass linear filter (i.e., a resistor-capacitor circuit), so that

$$K_m(t) = \frac{R}{\tau} e^{-t/\tau}$$

The electrode kernel is supposed to decay much faster, so that for large t, $K(t) \sim K_m(t)$. This suggests the idea of estimating K_m by fitting an exponential function to the tail of K and subtracting it ($K_e = K - K_m$).

However, a more careful examination of the circuit shows that the assumption $K = K_m + K_e$ is a crude approximation. Indeed, the recorded potential can be written as

$$\begin{aligned} V_r &= V_m + U_e \\ &= V_0 + K_m * I_m + K_e * I \end{aligned}$$

where V_m is the membrane potential (which is the quantity we want to recover), U_e is the potential across the electrode, and I_m is the current entering the membrane. The electrode filters the command current I; a reasonable approximation is to set $I_m = U_e/R_e$, where $R_e = \int K_e$ is the electrode resistance (defined as the ratio U_e/I for a constant injected current I). It follows that the full kernel reads

$$K = K_m * \frac{K_e}{\int K_e} + K_e \tag{1}$$

Thus the membrane kernel cannot be simply subtracted from the total kernel. However we can still use the tail of K to estimate the membrane time constant.

Using the tail of the kernel to estimate the membrane time constant

We assume that the electrode kernel K_e decays faster than the membrane kernel, i.e., $K_e(t) = o(e^{-t/\tau})$. In fact, we expect that $K_e(t) \sim \alpha e^{-t/\tau_e}$ for large t, with $\tau_e < \tau_m$. For large t, we have:

$$\begin{aligned} K_m * \frac{K_e}{R_e} &= \frac{R}{R_e \tau} \int_0^t e^{\frac{s-t}{\tau}} K_e(s) ds \\ &\sim \frac{R}{R_e \tau} e^{-\frac{t}{\tau}} \int_0^{+\infty} e^{\frac{s}{\tau}} K_e(s) ds \end{aligned}$$

where the convergence of the integral is guaranteed by the dominated convergence theorem. Thus, fitting an exponential function to the tail of the kernel gives the correct membrane time constant, but not the correct membrane resistance (it overestimates the resistance). For example, for a simple RC electrode with resistance R_e and membrane time constant τ_e, we obtain

$$K_m * \frac{K_e}{R_e} = \frac{R}{\tau} e^{-\frac{t}{\tau}} \times \frac{\tau}{\tau - \tau_e}$$

It follows that the membrane resistance is overestimated by about $\frac{\tau_e}{\tau}$ (with $\tau_e << \tau$). Thus for a (very) good recording the error would be around 1%.

How to define the "tail" of the kernel?

The result above is an equivalent when t goes to infinity. In practice, we need to split the kernel K at some point T (the tail parameter) and to fit an exponential function to the right part (the "tail" of the kernel). The larger this point T is, the closer the tail is from the correct exponential function ($e^{-t/\tau}$), but the fewer data points are available for the fitting procedure. Thus, there is a compromise in the choice of T. A rule of thumb is that T must be at least 10 times the expected electrode time constant, while allowing the tail to contain at least one membrane time constant. Typical values in our experiments were $T \approx 3 - 5$ ms. Supplementary Figure 1 shows that only the magnitude of T is important, the procedure is not very sensitive to the precise value.

Removing the membrane kernel

We use equation (1) to extract the electrode kernel K_e from K. Here we assume that the membrane kernel K_m has already been recovered, i.e., the parameters R_m and τ_m are known. In the next section we explain how to obtain good estimates for these parameters, but for the moment we can assume that we have obtained the correct parameters.

First, we need to estimate the electrode resistance. We have $R_e = \int_0^{+\infty} K - R_m$. In practice only the first M steps of the kernel is known, so that the formula

we need is actually:

$$R_e = \int_0^{M\Delta} K - R_m + R_m^* e^{-\frac{M\Delta}{\tau_m}}$$

where Δ is the sampling step and R_m^* is the estimate from fitting an exponential function to the tail of the full kernel K.

Then we use the Z-transform to solve equation (1). Since convolutions are transformed into products, we obtain:

$$\mathcal{Z}[K_e] = \mathcal{Z}[K] \left(\frac{\mathcal{Z}[K_m]}{R_e} + 1) \right)^{-1}$$

We have

$$\mathcal{Z}[K_m] = R_m \frac{\Delta}{\tau_m} \frac{1}{1 - \lambda z^{-1}}$$

with $\lambda = e^{-\Delta/\tau_m}$. We define $\alpha = \frac{R_m \Delta}{R_e \tau_m}$ and after a little algebra, we obtain

$$\mathcal{Z}[K_e] = \mathcal{Z}[K] \left(1 - \frac{\alpha}{\alpha + 1} \frac{1}{1 - \frac{\lambda}{\alpha+1} z^{-1}} \right)$$

The second term corresponds to a first order low-pass filter which can be implemented recursively as follows:

$$\begin{cases} Y_0 = \frac{\alpha}{\alpha+1} K_0 \\ Y_n = \frac{\alpha}{\alpha+1} K_n + \frac{\lambda}{\alpha+1} Y_{n-1} \quad \text{for } n > 0. \end{cases}$$

then $K_e = K - Y$.

Optimizing the membrane parameters

The difficulty in using the procedure above is that only τ_m can be estimated from the tail of the kernel K, while it is hard to estimate R_m reliably. If follows from section 5.4 that if R_m is not estimated correctly, then the estimated electrode kernel K_e includes a residual slow component (e^{-t/τ_m}) from the membrane kernel. Therefore we use the following strategy to obtain a better estimate of R_m: for each value R_m^* of the membrane resistance, the procedure gives an estimate of the electrode kernel $K_e(R_m^*)$; for the true value $R_m^* = R_m$ we expect the residual slow component to vanish, so that we search the resistance value which minimizes the tail of $K_e(R_m^*)$:

$$R_m = \underset{R_m^*}{argmin} \int_T^{+\infty} K_e(R_m^*)^2 dt$$

Since the variable to be adjusted is only one-dimensional, we simply use the golden search algorithm to find the optimal resistance. Note that the formula above is exact in the limit of large T.

5.5 Implementation

The computer implementation should follow easily from the algorithms we have previously described. In this section we outline a few important points and the general procedure. The programs must run on a real time computer system connected to the amplifier.

Estimation procedure

The estimation procedure lasts about 10 s and must be performed when the electrode is impaled in the neuron (because the properties of the electrode are not the same as in the extracellular medium). During this time, a uniform white noise current (in the form of a sequence of independent random numbers) is injected in the neuron. The signal is sent through an acquisition board to the amplifier. The amplifier should be properly set, with the capacitance neutralization circuit set at a high level (so as to reduce the time constant of the electrode). The bridge compensation circuit must be off. The range of the uniform noise must be the same as the range of the D/A converters of the acquisition board. The range of the input A/D converters, which relay the voltage recording to the computer, must be large enough to avoid clipping (it is best to check on an external oscilloscope). Although the membrane potential does not vary much, the electrode voltage is much more variable. For example, if the range of the uniform current is ± 1 nA and the electrode is very fast (i.e., faster than the acquisition rate) and its resistance is 100 MΩ (sharp electrode), then the potential would vary between -100 mV and 100 mV. Note that it can be useful to change the offset of the voltage output of the amplifier so that the resting potential is close to 0 mV.

The computer program does not need to store the whole sequence of measures (I and V). It is enough so store in memory the running averages of $I_n I_{n-i}$, $V_n I_{n-i}$, I_n and V_n. At the end of the stimulation, the program applies the Levinson-Durbin algorithm to find the full kernel and extract the electrode kernel with the algorithms described previously (exponential fitting of the tail followed by suppression of the membrane kernel). This part of the algorithm is not required to run in real time. Subsequently, only the electrode kernel needs to be stored. Typically, the resulting kernel is short and only the first tens of steps are non zero.

Online compensation

Once the electrode kernel has been calculated, it can be used in real time to estimate the electrode voltage and subtract it from the recording. Again, the bridge compensation circuit must be turned off on the amplifier. Then it must be remembered that the potential actually recorded by the system is the sum of the membrane and the electrode responses and therefore it can be much larger than the membrane potential. The electrode voltage is subtracted in real time by a convolution, the

input current I being known:

$$V_m(n) = V_r(n) - \sum_{p=0}^{l-1} K_e(p) I(n-p)$$

where l is the number of steps in the electrode kernel (typically 30–50). Thus, the value of the previous l steps of the injected current must be held in memory.

6 Typical sources of error

Here we enumerate a number of typical mistakes that can occur during the estimation or the compensation stage. Many of these errors can be easily noticed as anomalies in the electrode kernel.

The bridge compensation is on: in this case the program can still capture a kernel but it has a strange shape with a total resistance close to 0 (if it is well adjusted), which makes the membrane suppression procedure fail.

Input or output ranges on the acquisition board are not correctly set: if the ranges are too large, the method only loses some accuracy; however if the ranges are not large enough, then clipping occurs, which can be disastrous both at the estimation stage and at the compensation stage. It can remain unnoticed at the estimation stage because it only results in errors in the estimated kernel. At the compensation stage it results in large compensation errors which can be seen as noise on the compensated output in current clamp. It is more serious in dynamic clamp because it can result in losing the cell because of oscillatory instabilities.

The kernel is too large: if the number of steps M in the full kernel is very large, then during the estimation procedure the program may not have enough time to compute all the running averages within one time step. Depending on the real time system, this can result in freezing the program or in errors in the kernel. The latter is more problematic because it can remain unnoticed: in this case, the program sometimes takes more than one step to do all the required operations and it can be an important source of error. Therefore it is important to check that the kernel is not too large for the system.

The tail parameter is too small: one must specify what part of the kernel (which we called the tail) is used for estimating the membrane contribution. If the splitting time is too small, then the tail contains part of the electrode kernel, which makes the procedure fail. This can sometimes be seen as a negative part appearing in the electrode kernel (usually the kernel is entirely positive).

The tail parameter is too large: if the splitting time is too large, then remaining tail is too small to estimate the membrane kernel reliably. This also results

in errors in the electrode kernel (although not as serious). There is however a broad range of values of this parameter for which there is no significant error in the kernel (Supplementary Figure 1).

The capacitance neutralization has changed: it must be remembered that the electrode kernel captures in fact not only the electrode properties, but the properties of the whole recording setup, including the amplifier. Therefore if any circuit is used on the amplifier, their setting must remain unchanged as long the same electrode kernel is used, otherwise the estimation procedure should be run again.

The electrode properties have changed: it happens that the electrode properties change during an experiment for some reason (e.g. small movements of the electrode). It results in compensation errors which can be seen as abnormal noise on the traces (with current noise injection). In this case the estimation procedure must be run again (just like with the standard bridge compensation method). The best practice is to run the estimation once in a while in order to check that the electrode properties have not changed.

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