DYNAMIC CLAMP WITH HIGH RESISTANCE ELECTRODES USING ACTIVE ELECTRODE COMPENSATION IN VITRO AND IN VIVO

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ABSTRACT

The Active Electrode Compensation (AEC) consists of an online correction of the recorded membrane potential based on a computational model of the electrode. This technique may be particularly useful for situations where high-frequency components (such as noise) must be injected. This is particularly important for dynamic-clamp applications because of the real-time feedback between injected current and recorded voltage, since any artifact is amplified and may cause instabilities. We show here that such problems are greatly limited by the AEC, and this technique enables dynamic-clamp injection at high feedback frequencies (>10 KHz) and in demanding conditions. We illustrate applications such as injection of conductance noise *in vivo* and *in vitro*.

1. INTRODUCTION

1.1. DYNAMIC CLAMP WITH A SINGLE HIGH-RESISTANCE ELECTRODE

The dynamic-clamp, or "conductance injection" (Robinson and Kawai, 1993; Sharp et al., 1993; Prinz et al., 2004), consists of injecting a current $I(t)$ that depends on the value of the membrane potential $V(t)$, according to Ohm's law $I(t) = g(t)\,[V(t)-E]$, where $g(t)$ is the conductance injected and E is the reversal potential. In many situations, especially *in vivo*, current injection and voltage measurement are performed with the same electrode. *In vivo*, this electrode is either a high resistance sharp microelectrode with low capacitance (Steriade et al., 2001; Wilent and Contreras, 2005a; Crochet et al., 2006; Higley and Contreras, 2007; Haider et al., 2007; Paz et al., 2007) that are also used in some adult *in vitro* preparations (Thomson and Deuchars, 1997; Shu et al., 2003), or a patch electrode that can display a whole range of resistances and capacitances depending on the age and species of the animal (Margrie et al. 2002, Borg-Graham et al., 1998,

Monier et al. 2008, Pei et al. 1991, Hirsch et al. 1998, Anderson et al. 2000, Wehr and Zador, 2003, Mokeichev et al., 2007). The problem inherent to such single-electrode recordings is that the injected current biases the measurement because of the voltage drop through the electrode.

In a typical current-clamp recording with a single electrode, where current is simultaneously injected, the recorded potential is $V_r=V_m+U_e$, where V_m is the membrane potential (which is the variable we are interested in) and U_e is the voltage across the electrode. As a first approximation, the electrode acts as a resistance: $U_e \approx R_e I$, where R_e is the electrode resistance and I is the injected current. Thus a first estimation, known as *bridge compensation*, is $V_m \approx V_r - R_e I$. However, this is too crude an approximation because the electrode has a non-zero charge time and is better modeled by a RC circuit (resistance + capacitance). Amplifiers include a capacitance neutralization circuit which amounts to inserting a negative capacitance in the circuit (Thomas, 1977). Again, this model is too simple because there is always a residual capacitance, which appears as capacitive transients in recorded responses to current pulses (Fig. 1a). In dynamic clamp, the artifacts are injected back and can be amplified by the control loop, which leads to oscillatory instabilities (Fig. 1b; see Appendix B for a mathematical analysis). An option is to use a discontinuous mode (Fig. 1c), alternatively injecting current and recording the V_m (Brennecke and Lindemann, 1971, 1974a, 1974b; Finkel and Redman, 1984) with a frequency set by the electrode time constant (typically 1.5-3 kHz with sharp electrodes in our experiments in cortical neurons *in vitro* and *in vivo*). Unfortunately, the alternation method is valid only when the electrode response is at least two orders of magnitude faster than the recorded phenomena (Finkel and Redman, 1984), because the membrane response must be quasi-linear in the sampling interval. Moreover, recordings in discontinuous modes are very noisy and sampling frequency is limited, which makes the precise recording of fast phenomena like spikes impossible (Fig. 1d).

We developed a method named Active Electrode Compensation (AEC) based on a digital model of the electrode seen as an unknown linear filter, which allows sampling the V_m during current injection with a frequency only limited by the speed of the computer simulating the electrode model (Brette et al, 2007, 2008). In this chapter, we review this method and its application to dynamic-clamp experiments.

1.2. ACTIVE ELECTRODE COMPENSATION

The AEC method consists in using a more complex model of an electrode, by assuming that the electrode can be seen as an arbitrarily complex circuit of resistances and capacitances, which can be represented by a linear time-invariant filter, i.e., the response of the electrode to a current $I(t)$ is expressed as a convolution:

$$U_e(t) = (K_e * I_e)(t) = \int_0^{+\infty} K_e(s) I_e(t-s) ds$$

where $K_e(\cdot)$ is named the *electrode kernel* (Fig. 1e,f). Thus the voltage across the electrode depends linearly on all past values of the injected current. This formulation encompasses any linear model, e.g. a circuit with a resistor and a capacitor (the kernel K_e is then an exponential function). The technique consists in identifying the electrode kernel by observing the response of the electrode to a known noisy current. In practice, the electrode kernel can only be estimated when the electrode impales the neuron (because electrode properties change after impalement).

In this case, we first remove the membrane kernel from the full measured kernel (see next section).

Once the electrode kernel has been estimated, we use the expression above to estimate the voltage across the electrode during current injection and subtract it from the recording $V_r(t)$. In practice, the recordings are digitized and the formula reads:

$$U_e(n) = \sum_0^{+\infty} K_e(p) I_e(n-p)$$

and the digital convolution is performed in real time by a computer.

We first describe the implementation of AEC in detail (section 2), then we examine the influence of various aspects on AEC recordings, such as time constants and electrode nonlinearities (section 3) and finally we show some practical examples of recordings *in vitro* and *in vivo* (section 4). The appendixes contain more details about typical errors using AEC (A) and dynamic clamp instabilities with standard bridge compensation (B). Sample code is available at the following URL: http://www.di.ens.fr/~brette/HRCORTEX/AEC/.

2. THEORY AND IMPLEMENTATION

The algorithm to estimate the electrode kernel (Fig. 2) consists in 1) finding the kernel of the full system neuron + electrode (+ amplifier) from the voltage response to a known input current, and 2) extracting the electrode kernel form the full kernel (see Fig. 2). Indeed, the electrode kernel cannot be measured in isolation because the electrode properties change after it penetrates the membrane. Once the electrode kernel is measured, the electrode response can be calculated online by convolving the kernel with the injected current; then it is subtracted from the voltage recording to obtain the membrane potential.

2.1. FINDING THE KERNEL

We assume that the neuron and the electrode respond linearly to the injected currents we use. We will discuss this hypothesis later (sections 3.4 and 3.5). 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), also named the *kernel*, and V_0 is the resting potential. In the digital domain, the formula reads

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

Note that both the continuous and the discrete formulas express the linearity of the response and $V(t_n)=V_n$, but the continuous and discrete kernels are generally not identical (they only agree in the limit of small sampling steps). In fact, the kernel contains not only the neuron and

the electrode, but also everything else that is between the output of the computer and the electrode, including all the filters and circuits in the amplifier (e.g. the capacitance neutralization circuit).

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 (Δ=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 **AX**=**B**, where **A** is a square matrix with coefficients $a_{i,j} = \langle I_{n-j} I_{n-i} \rangle$ for $i,j \in \{0 \ldots M-1\}$, $a_{i,M} = \langle I_{n-i} \rangle$ for $i \in \{0 \ldots M-1\}$, $a_{M,j} = \langle I_{n-j} \rangle$ for $j \in \{0 \ldots M-1\}$ and $a_{M,M} = 1$; **X** is a column vector with $X_i = K_i$ for $i \in \{0 \ldots M-1\}$ and $X_M = V_0$; **B** is a column vector with $B_i = \langle V_n I_{n-i} \rangle$ for $i \in \{0 \ldots M-1\}$ and $B_M = \langle V_n \rangle$. Solving this linear equation for **X** gives the coefficients of the kernel K and the resting potential V_0.

Although there is no theoretical problem in solving the linear problem described above, the matrix **A** can be large and each coefficient 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 **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 $n \in \{N-M+1 \ldots N-1\}$, 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 $i \in \{0 \ldots M-1\}$. It follows that the matrix has a special form known as a *Toeplitz matrix*, and solving a linear problem for a such a matrix can be done very quickly with the use of the Levinson-Durbin algorithm (which is documented for example in Press et al, 1993). Besides, it is not necessary to store all the values of V_n and I_n, since the averages $\langle V_n I_{n-j}\rangle$ can be computed online in real time (M additions at each time step). More details about expressing the problem with a Toeplitz matrix can be found in Appendix C.

2.2. CHOOSING THE INPUT SIGNAL

The input signal I must be chosen so that

1. it is zero at the end of the stimulation (last M steps, where M is the kernel size) in order 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.

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

MEMBRANE RESPONSE DURING INJECTION

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 electrode. 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 voltage 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, 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; see Fig. 5e,f and Fig. 7a).

CHOOSING 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 Δ=0.1 ms, τ_m=10 ms, R_m=40 MΩ and $I_{\max}$=0.5 nA, we obtain σ_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 has the same magnitude 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.

2.3. 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 more precisely 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 Re is the electrode resistance (defined as the ratio U_e/I for a constant injected current I). It follows that the full kernel reads

$$K = K_m * \frac{K_e}{\int K_e} + K_e \qquad \textbf{(*)}$$

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. Indeed, if $K_e(t)=o(e^{-t/\tau})$ with $\tau_e<\tau_m$, then 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).

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 choice and sensitivity to this parameter is discussed in section 3.1 (typical values in our experiments were T≈3–5 ms).

REMOVING THE MEMBRANE KERNEL

We use equation (*) 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 are known, so that the formula we need is actually:

$$R_e = \int_0^{M\Delta} K - R_m + R_m^0 e^{-M\Delta/\tau_m}$$

where Δ is the sampling step and R_m^0 is the estimate from fitting an exponential function to the tail of the full kernel K (which would be the first guess for R_m). Once R_e and K_m are known, we invert the relationship (*) by using the Z-transform (see Appendix D – in the end it simply amounts to applying a low-pass filter).

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 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 can 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. In some practical cases when the electrode resistance is very small compared to the membrane resistance (typically with patch electrodes), there can be several local minima and the golden search algorithm can lead to the wrong value. A simple way to avoid this problem is to isolate the first minimum (which is the correct one) by calculating the error measure for a small value of $R_m^*$and increase R_m^* in logarithmic steps (i.e., multiplying by a constant factor at every step) until the error starts to grow; then the first minimum is bracketed by the last two values of R_m^*.

2.4. 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 (using for example a circular array).

3. PRACTICAL ASPECTS

The quality of AEC recordings depends on a number of parameters and assumptions. In this section we examine the influence of AEC parameters such as the duration and amplitude of the white noise injection (3.1), the instabilities that may arise from the feedback delay of the acquisition system (3.2), and the robustness of the method with respect to the main three hypotheses we made: the electrode time constant is short compared to the membrane time constant (3.3), the membrane response is essentially exponential (3.4) and the electrode is linear (3.5). In practice, these hypotheses are only approximations but fortunately it generally has a mild effect on the quality of electrode estimation.

3.1. ESTIMATION PARAMETERS

The kernel estimation algorithm relies essentially on the following parameters: the parameters of the noise injection (duration and amplitude) and the parameters of the kernels (size and the tail parameter).

In theory, in a noise-free system, observing the response to a current injection with same duration as the kernel is enough to determine the kernel, that is, about 10-20 ms. However real systems are noisy and recording noise can be averaged out by using longer durations. We found (using high-resistance sharp electrodes) that 5 s was enough to obtain reliable and smooth kernels *in vitro* (we found no difference with 10 and 20 s stimulations; see Fig. 3c,d). A shorter duration is generally sufficient but 5 s is safer (in one case we found a significant difference with an injection lasting only 1 s). *In vivo*, recordings are noisier because of the network activity; in our experience, reliable estimates could be found with stimulations lasting 20 s. The amplitude of the noise current should be set so that the membrane response is small, as explained in section 2.2; we found no significant impact of this parameter in the experiments. A constant current can also be applied in addition to the white noise, for example to avoid spikes. In this case the kernel estimation can be sensitive to the amount of DC current if the electrode is significantly nonlinear. This issue is addressed in detail in section 3.5.

The size of the kernel (neuron + electrode) is essentially determined by the speed of the hardware, since the computational cost of online operations is directly proportional to the number of values in the kernel. We used 150-200 points with a 10 kHz sampling rate (i.e., the kernel size was 15-20 ms). A rule of thumb is that the kernel should contain about one membrane time constant (although this is not a crucial requirement). In fact, the kernel size should not be too large (i.e., many times the membrane time constant), otherwise a large part of the kernel is non-significant (close to zero) and dominated by noise.

The tail parameter is used for extracting the electrode kernel; it corresponds to the time from which the full kernel is considered as corresponding to the membrane response only, and it has to be chosen prior to the separation of the full kernel into a membrane kernel and an electrode kernel. The membrane time constant is estimated from the tail of the kernel and the electrode kernel is assumed to vanish from that point. There is clearly a trade-off in the choice of this parameter: it must be large enough so that the electrode kernel does indeed vanish after that point, but small enough so that there are enough remaining points to estimate the membrane time constant. Fortunately, there is a broad plateau of parameter values for which the kernel estimation is correct (see simulation results in Fig. 3a, confirmed by *in vitro* experiments in Fig. 3b).

3.2. FEEDBACK DELAY

In a digital dynamic clamp operated by a computer (as opposed to an analog dynamic clamp system), the feedback delay is a source of instability – which is not specific to AEC. A computer or a digital signal processor records and injects at sampling rate f. At time $t_n=n/f$, the membrane potential $V_n=V(t_n)$ is sampled, then the computer calculates the current to inject $I_n=g(E-V_n)$ during the interval $[t_n, t_{n+1}[$, and the current is injected during the next interval $[t_{n+1}, t_{n+2}[$ (hence the feedback delay is $2/f$). Assuming perfect electrode compensation (i.e., in effect, no electrode), the dynamics of the sampled membrane potential is given by the following recurrence equation:

$$\begin{aligned} V_{n+2} &= \lambda V_{n+1} + (1-\lambda) R I_n \\ &= \lambda V_{n+1} + (1-\lambda) gR(E-V_n) \end{aligned}$$

where $\lambda=\exp(-1/\tau f)\in]0,1[$ ($\tau=RC$ is the membrane time constant). In general the sampling step is at least two orders of magnitude smaller than the membrane time constant, so that $\lambda\approx 1-1/(\tau f)$. This is a second order linear recurrence equation, and the solutions are determined by the roots of the polynomial $X^2-\lambda X+(1-\lambda)gR$. Oscillations can arise if the discriminant is negative, i.e., $\lambda^2-4(1-\lambda)gR<0$, which is approximately (using $1/f<<\tau$):

$$gR > \frac{\tau f}{4}$$

In this case the real part of the roots is $-\lambda/2$, which is smaller than one, so that the solution of the recurrence equation is a damped oscillation, i.e., ringing. If the discriminant is positive, then there are two real roots a and b, such that $a+b=\lambda\in]0,1[$ and $ab=(1-\lambda)gR>0$. The latter inequality means that a and b have the same sign, and $a+b>0$ implies that this sign is positive. From the inequality $a+b<1$, it follows that a and b are both in $]0,1[$, therefore the solutions of the recurrence equation are stable.

Thus, in the case of perfect compensation, the feedback delay induces ringing if $gR>\tau f/4$ but does not destabilize the system for constant conductances. In our experiments, $\tau f\approx 100$, so that the maximum clamp conductance is about 25 times the membrane conductance.

Note that this inequality also applies to the maximal feedback gain of a digital voltage-clamp system (the expression we obtained is very similar to the formula derived by Finkel and Redman (1984) in the analysis of the discontinuous voltage-clamp: $g_c=\tau f/(RD)$, where D is the duty cycle).

3.3. TIME CONSTANTS

To separate the membrane kernel and the electrode kernel, we use the assumption that the electrode kernel is shorter than the membrane kernel, so that the membrane time constant can be estimated from the tail of the full kernel. This estimation can still be done if the electrode time constant is only slightly shorter than the membrane time constant. However, the quality of the electrode kernel estimation degrades with larger ratios τ_e/τ_m, so that the electrode should be as fast as possible (this is one reason why it is still useful to use the capacitance neutralization circuit on the amplifier – which is a feedback rather than a compensation method). Numerical

simulations (Fig 4a) show that the quality degrades continuously with larger ratios and starts to degrade seriously when this ratio is greater than 1/10 (the rule of thumb is that the error in signal reconstruction grows as τ_e/τ_m), so that AEC should be useful when the electrode time constant is about one order of magnitude shorter than the membrane time constant (in our somatic recordings with sharp microelectrodes, the electrode time constant was about 0.1 ms, so that the minimum membrane time constant for a good kernel estimation should be about 1 ms).

When the electrode time constant is large, the quality of recordings is impaired but the technique is still usable (in continuous mode), as shown on Fig. 4b-d (numerical simulations), where the ratio of time constants is about 1/10. The situation is quite different with DCC, which also has a constraint on time constants: proper operation of the DCC requires ratios lower than 1/50-1/100, but the technique does not give a meaningful signal if the ratio is higher (first because the optimal clock frequency is very low, second because it is impossible to accurately determine the optimal frequency).

All single-electrode compensation methods have a requirement on the ratio of time constants (τ_e/τ_m): setting the bridge resistance requires separating electrode and membrane responses, while the discontinuous current clamp is valid when the ratio is about 1/100 or better. In this respect, AEC has a better critical ratio, so that it can be used in more situations than either bridge or DCC.

3.4. DENDRITES

To extract the electrode kernel from the full kernel (neuron + electrode), we assume that the membrane kernel is a single exponential function and estimate the time constant from the tail of the kernel. In fact, due to the dendritic tree, there are additional exponential modes with faster time constants, some of which are similar to the electrode time constant, as shown in Fig. 5 (simulation of a pyramidal cell). With a single electrode (for any method), there is no way to distinguish fast dendritic contributions from electrode contributions at the same time scale. In fact, for any linear time-invariant system, such as a linear electrode and a passive neuron with a dendritic tree, the relationship between the current injected at a given point and the potential recorded at another (or the same) point is fully characterized by the kernel, so that there is no more information that we may have on the system. Therefore, an electrode compensation system must rely on the fact that the fast dendritic contributions to the kernel are relatively small in amplitude. This might not be always the case (and if so, a second electrode would be necessary), but at least in numerical simulations of morphologically reconstructed pyramidal cells, the dendrites contribute not more than a few MΩ of the total resistance (the resistance of typical sharp electrodes are around 80 MΩ).

Because the fast dendritic contribution to the kernel is included in the electrode kernel, and therefore subtracted by electrode compensation, there is a little less high-frequency power in the compensated trace than in the real membrane potential. However, this is a constant subtraction and not a low-pass filtering, so that fast active changes in membrane properties (e.g. spikes) are not filtered out by the method, as shown in Fig. 5f.

3.5. NONLINEARITIES

The crucial hypothesis underlying the AEC technique is that the electrode is linear. However, it is known that high-resistance electrodes can be nonlinear, which is characterized by the fact the electrode resistance can change with strong currents. Physical modeling of nonlinearities (Purves, 1981) indicates that these are slow processes due to redistribution of ions near the

electrode tip. Nonlinearities are stronger for electrode tips with a small radius (which is inversely correlated with the resistance) and when the concentrations of the two solutions (intracellular and inside the electrode) differ. However in practice the amount of electrode nonlinearity is highly variable and unfortunately cannot be assessed before the electrode is impaled into the cell – although electrodes with an unusually high resistance in the slice can be discarded from the start. This nonlinearity problem is not different with AEC than with standard bridge compensation, however AEC provides a simple way to measure it, and possibly discard the recordings if the nonlinearity is too important. We first note that nonlinearities are too slow to affect the stability of dynamic clamp recordings, so that the issue is rather the quality of voltage estimation.

Electrode nonlinearities are usually measured before impalement from the I-V curve of the electrode, but it is not possible to use the same approach intracellularly because the I-V curve of the electrode could be confused with the I-V curve of the neuron. AEC can be used to measure the electrode resistance by running the kernel estimation procedure intracellularly with different levels of constant injected current (Fig. 6), corresponding to the typical (average) levels that will be used subsequently, and check that the amount of nonlinearity is acceptable (in our experiments, about half the electrodes were not significantly nonlinear). This raises the issue of estimating the kernel when the membrane has a nonlinear, possibly spiking, response. Although in principle, the response of the whole system (membrane + electrode) should be linear during the estimation of the electrode kernel, this is not a strong requirement for the membrane, because the method calculates the best linear approximation to that response. The full kernel is, to a first approximation, Ke + Km, and if the membrane behaves non-linearly (e.g. spiking), the impact will mainly be on Km, therefore the main issue in that case is whether the estimated Km differs significantly from an exponential function, so that it can be properly subtracted. Numerical simulations show that moderate spiking activity has a small impact on kernel estimation; we were also able to estimate electrode kernels during moderate spiking activity in vivo (see next section).

In dynamic clamp, the injected current can be transiently very high: for example during an action potential, the injected current becomes typically very large and negative (for example for a current of the form $I=g(E-V_m)$ where E is low). These transients would be a big problem for electrode compensation if nonlinearities were fast. Fortunately, we confirmed that transients do not trigger significant nonlinearities. Fig. 6e shows the measured compensated voltage and current at the peaks of action potentials during conductance noise protocols (*in vitro* and *in vivo*). Those two quantities were very weakly correlated (the slopes of linear regressions ranged from -.1 MΩ to -.6 MΩ), which indicates that the electrode resistance could not have changed much during spikes (any error ΔRe in the estimation of the electrode resistance would result in an error ΔRe*I in the voltage estimation.).

Electrode nonlinearity can be approximated by a quadratic dependence of voltage on current or equivalently as a linear change in resistance with injected current (MΩ/nA). The voltage error resulting from an error ΔRe in the estimation of the electrode resistance can be described simply as follows (see Fig. 6d). The resistance error (compared to infinitesimal current injection) is $\Delta Re = \lambda * I$, where λ (in MΩ/nA) quantifies the nonlinearity (measured with AEC as in Fig. 6a-c). If the membrane resistance is Rm, then the voltage error resulting from the nonlinearity is $\lambda * I^2 = (\lambda/Rm^2) * \Delta V^2$, where ΔV is the depolarization of the membrane induced by the injection (= Vm - resting potential). For example, if the electrode nonlinearity is 2 MΩ/nA and the membrane resistance is 60 MΩ, then the voltage error is 0.0005 mV / mV^2 (e.g. 0.5 mV for 30 mV depolarization). This analysis applies to linear depolarizations induced by constant subthreshold currents, not for example for action potentials, which are transient.

4. AEC RECORDINGS IN VITRO AND IN VIVO

PRACTICAL ISSUES

There are two main important practical issues in experiments, especially *in vivo*: whether the electrode kernel can change over the time course of an experiment, and whether the technique can be easily used given unavoidable perturbations such as recording noise and synaptic activity.

It is known that the electrode resistance can change from time to time over the time course of an experiment. It is then necessary to recalibrate the technique (e.g. balance the bridge). The same applies for AEC, but not more often than with other techniques. In practice, one should run the estimation procedure from time to time and check that the kernel has not changed. Fortunately, it only takes a few seconds and it is fully automatic. *In vivo*, we found that electrode properties could remain stable for up to two hours, as assessed with kernel estimations obtained repetitively, also when using different constant current injections and different durations of white noise injection (Fig. 7b). Regarding stability, an important point is that AEC allows the use of high-resistance sharp electrodes, which are more stable *in vivo* than patch electrodes.

The second practical issue is the problem of ongoing synaptic activity and recording noise. Recording noise is not a very big problem for the technique because the number of measurements (50,000 points for 5 s estimations at 10 kHz) is much larger than the number of points in the kernel (typically around 150), so that noise is averaged out. Important variations of the membrane potential during estimation, such as spiking activity, could be more problematic. However, as we mentioned earlier, the linearity hypothesis is crucial for the electrode but not so much for the membrane, because the method finds the best linear kernel and the membrane part is removed – the fact that the membrane kernel is not accurate is not an issue as long as the electrode kernel is correctly recovered. Membrane nonlinearities can affect the electrode kernel only if they introduce a systematic current-voltage relationship at the time scale of the electrode; otherwise the perturbations are averaged out. We checked in numerical simulations that electrode kernel estimation is possible during spiking activity, with a small impact on the estimated kernel. We were indeed able to run the procedure *in vivo* while the neuron was spiking and use the resulting electrode kernel for subsequent dynamic clamp experiments (Fig. 7a). *In vivo*, we used longer injections (20 s) to make sure that all sources of noise are averaged out.

EXAMPLES

Here we show a number of examples of dynamic clamp experiments using AEC *in vitro* and *in vivo*.

SQUARE CONDUCTANCE WAVES

We injected a square wave of alternating "excitatory" ($E_{excitation} = V_{rest} + 10$) and "inhibitory" ($E_{inhibition} = V_{rest} - 10$) conductance pulses with different conductance amplitudes (range 10-100 nS) and frequencies (range 10-1000 Hz) *in vitro*, with sharp electrodes (Fig. 7c). This is a challenging protocol if the conductance or the frequency is large. In particular, bridge compensation can be used only for very small conductances (see Appendix B), and DCC shows

strong artifacts at high frequencies. The responses with AEC correspond to what would be expected from a passive cell model.

CONDUCTANCE NOISE

We injected a colored conductance noise consisting of two stochastic variables, $g_e(t)$ for excitation and $g_i(t)$ for inhibition (Figure 7d), mimicking cortical synaptic background activity as seen *in vivo* (Destexhe et al., 2001). This protocol could not be performed with bridge compensation (recordings are unstable). With AEC we could easily inject synaptic noise of high total conductance (5 times the leak conductance). The spikes can be recorded with excellent precision (while they would be reduced to a few sampling points with DCC). We performed this protocol both *in vitro* and *in vivo*.

RELIABILITY OF SPIKE TIMING WITH RECREATED SYNAPTIC ACTIVITY

The precision of dynamic clamp recordings with AEC allowed us to evaluate the reliability and precision of spike timing in cortical neurons with recreated synaptic activity, instead of current noise (Mainen and Sejnowski, 1995). Figure 7e shows the responses of cortical neurons to repeated dynamic clamp injections of frozen conductance noise, mimicking synaptic activity (as described above). In cortical neurons in vitro (left), the repetition of such a realistic, fluctuating conductance stimulus can lead to highly precise and reliable spiking patterns (as has also been shown by others, e.g. Harsch and Robinson, 2000; Tateno and Robinson, 2006). In a cortical neuron in vivo, however (right), due to real synaptic inputs from the network and possibly to different intrinsic properties of the recorded cell (note the Vm close to threshold at the beginning of the shown traces, before any conductance injection), the situation is more complex: on the example shown, one particular spike (box) appears as precise and reliable from trial to trial, but this is not the case for the other spikes. This example illustrates that future dynamic clamp experiments in vivo, enabled by the AEC method, would be useful in identifying the conditions for spike-timing precision and reliability in functioning cortical networks.

5. DISCUSSION

In this chapter, we have reviewed the recent developments of a technique to perform dynamic-clamp experiments at high resolution using single electrodes. We have described the theory behind AEC and its implementation (Section 2), as well as some practical aspects such as how to estimate compensation parameters, and the effect of electrode nonlinearities and of the dendrites (Section 3). We also reviewed practical examples of demanding conditions, such as injection of conductance noise and dynamic-clamp experiments *in vivo* (Section 4). We now discuss the AEC technique in reference to other compensation techniques, as well as future perspectives.

The AEC technique is based on a non-parametric linear model of the electrode which is automatically calibrated. It allows accurate intracellular recordings at a high sampling frequency during simultaneous current injection, uncontaminated by capacitive transients, which makes it especially appropriate for dynamic clamp protocols, especially with high-resistance electrodes. Previous techniques suffered from either instability issues (bridge compensation) or limited temporal resolution (DCC). Another advantage of model-based electrode compensation is that the technique provides the precise characteristics of the electrode along with the recording, which can be useful to estimate the recording quality. It should be noted that AEC compensates for the electrode voltage, but does not modify the injected current. Thus, the injected current

remains filtered by the electrode, although the true injected current can be approximately estimated off-line from the knowledge of the electrode kernel.

The main condition that has to be met for the method to work accurately is the linearity of the electrode and of the whole recording chain between the electrode and the computer (amplifiers and filters), for the range of expected currents. The technique provides a fast and automated way to measure electrode nonlinearities intracellularly, which was previously difficult, if not impossible. One can then estimate the resulting errors in subsequent recordings, and possibly discard the recordings if the nonlinearity is too important. These nonlinearities are due to slow processes, so that large currents that are only transiently injected should not degrade the quality of electrode compensation.

Another requirement, which is also shared by previous techniques, is that the electrode and membrane time constants should be well separated, which means that the capacitance neutralization provided by the amplifier should be used optimally like in the other methods. However, admissible results can be obtained if the electrode is only 10 times faster than the membrane, while DCC requires electrodes about 100 times faster than the membrane. Thus AEC should extend the applicability of single-electrode dynamic clamp to neurons with much shorter time constants.

We are now investigating several extensions of the AEC technique. In particular, we are currently working on using our compensation method for the single-electrode voltage-clamp. The development of this "AEC-VC" technique represents a considerable interest because it would enable recording in voltage-clamp mode using sharp electrodes and without using a discontinuous mode, therefore enabling continuous voltage-clamp recordings with sharp electrodes *in vivo*. We are presently testing this technique on sharp-electrode recordings *in vivo* and *in vitro*, and compare it with discontinuous voltage-clamp (DVC) methods. Other possible extensions include dendritic patch-clamp recordings which use electrodes with higher series resistance compared to somatic patch recordings, as well as applications of the AEC to model electrode nonlinearities.

APPENDIX

A) TYPICAL SOURCES OF ERRORS

Here we enumerate a number of anomalous situations that can occur during the estimation or compensation stages that may produce unwanted biases in the electrode kernel estimate. Many of these errors can be easily noticed as anomalies in the electrode kernel, as is illustrated in Fig. 8.

The bridge compensation is on: in this case the program can still capture a kernel but it has a strange shape (Fig. 8a) 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 (Fig. 8b). 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 the electrode kernel not vanishing at the end (Fig. 8c) or 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.

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 capacitance neutralization is too high: instability problems can appear if the capacitance neutralization circuit is set at a high value. This is not specific to AEC, but it can be identified in the electrode kernel as damped oscillations (Fig. 8d). The oscillations should disappear if the capacitance neutralization is set at a slightly lower value.

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.

The amplifier or filters are nonlinear: normally good electrical circuits in amplifiers and acquisition boards should be linear. However there can be small fast electrical nonlinearities if some components are faulty. This can be identified as small changes in the kernels for different noise amplitudes, as small transients in response to current pulses or as abnormal noise on compensated traces with fluctuating inputs (Fig. 8e,f). Searching for electrical nonlinearities should be done using an electronic circuit modeling the cell + electrode.

B) DYNAMIC CLAMP ERRORS WITH BRIDGE COMPENSATION

B.1. STEADY-STATE ERRORS RESULTING FROM POOR ELECTRODE COMPENSATION

At equilibrium, the recording potential before compensation is $V_r=V_m+R_eI$ (by definition of the electrode resistance, independently of its properties). If the estimated electrode resistance is $R_e+\Delta R_e$ (whether by bridge compensation or AEC), then the estimated potential is $V=V_m+\Delta R_eI=(R+\Delta R_e)g(E-V)$, thus

$$V = \frac{(R+\Delta R_e)gE}{1+(R+\Delta R_e)g}$$

which can be expressed as follows: the dynamic clamp interprets the residual electrode resistance as part of the membrane resistance. The membrane resistance is

$$V_m = RI = gR(E-V) = \frac{gRE}{1+(R+\Delta R_e)g}$$

Therefore the relative error (compared to the case $\Delta R_e=0$) is

$$\begin{aligned}\frac{V_m}{V_m^*}-1 &= \frac{1+gR}{1+(R+\Delta R_e)g}-1\\ &= -\frac{\Delta R_e g}{1+(R+\Delta R_e)g}\\ &\approx -\Delta R_e g\end{aligned}$$

(for small error ΔR_e). For large clamp conductance g, the relative error tends to $-\Delta R_e/(R+\Delta R_e)$.

B.2 INSTABILITIES WITH BRIDGE COMPENSATION

We consider a dynamic clamp protocol with ideal bridge compensation, i.e., $V=V_r-R_eI$, where R_e is the (perfectly estimated) electrode resistance. The dynamic clamp is analog, so that there is no feedback delay. The electrode is modeled as a resistor (R_e) and a capacitor (C_e). In the absence of the capacitor, the bridge estimation is perfect, i.e., $V=V_m$, otherwise it differs from the real membrane potential because of the capacitive current through the electrode. The dynamic clamp current is $I=g(E-V_r-Re_I)$, thus:

$$I = \frac{g(E-V_r)}{1-gR_e} = \alpha(E-V_r)$$

where α is a definition. We already note that $\alpha<0$ if and only if $gR_e<1$, which, as we will see, is the stability condition. In the following we consider Laplace transforms of the time-dependent variables. With the Laplace variable s, the impedance of a capacitor is $1/(Cs)$. The current flowing through the electrode is the command current minus the current flowing through the electrode capacitance:

$$I_e = I - C_e s V_r = \alpha(E - V_r) - C_e s V_r \qquad (1)$$

It also equals the transmembrane current:

$$I_e = (R_m^{-1} + Cs)V_m = (R_m^{-1} + Cs)(V_r - R_e I_e)$$

and thus

$$I_e = \frac{(R_m^{-1} + Cs)V_r}{1 + (R_m^{-1} + Cs)R_e} \qquad (2)$$

From equations (1) and (2), it follows:

$$(R_m^{-1} + Cs)V_r = (\alpha(E - V_r) - C_e s V_r)(1 + (R_m^{-1} + Cs)R_e)$$

The solutions are stable if and only if the roots of the following polynomial have a negative real part:

$$R_m^{-1} + Cx + (\alpha + C_e x)(1 + (R_m^{-1} + Cx)R_e)$$

This is equivalent to the statement that the sum of the roots is negative and their product is positive, that is:

$$\begin{aligned} C(\alpha R_e + 1) + C_e(1 + R_m^{-1} R_e) &> 0 \\ R_m^{-1} + \alpha(1 + R_m^{-1} R_e) &> 0 \end{aligned}$$

If $\alpha>0$, this is clearly true. Conversely, the second inequality can be re-expressed as $1+\alpha(R_m+R_e)>0$, and using the formula for α:

$$\frac{1 + gR_m}{1 - gR_e} > 0$$

which is true if and only if $gR_e<1$ (i.e., $\alpha>0$).

Thus, the condition for stability with ideal bridge compensation is $gR_e<1$. There is no ringing in this case (only non-oscillatory instability), it only occurs when feedback delays are introduced (see next paragraph). To compare with the stability condition in the next section, it is useful to write this condition as follows:

$$gR < \frac{R}{R_e}$$

In our experiments (with high resistance electrodes), this ratio was between 0.3 and 1, and in that case only conductances smaller than the membrane conductance can be injected. When the

electrode is correctly compensated, the limiting factor is the feedback delay, as explained below, and the critical clamp conductance is much higher.

In the derivation of the critical conductance, we modeled the electrode resistance as a simple RC circuit, with the capacitor on the amplifier side. If the capacitance is distributed, the result would change slightly; for example if the capacitor is moved to the middle of the resistor, then the critical conductance will be twice higher (because half of the resistance if fully compensated).

C. FAST IMPLEMENTATION OF KERNEL ESTIMATION

In section 2.1, we saw that the least square estimation of the kernel is a matrix problem **AX**=**B**, where **A** is a square matrix with coefficients $a_{i,j} = \langle I_{n-j} I_{n-i} \rangle$ for $i,j \in \{0 \ldots M-1\}$, $a_{i,M} = \langle I_{n-i} \rangle$ for $i \in \{0 \ldots M-1\}$, $a_{M,j} = \langle I_{n-j} \rangle$ for $j \in \{0 \ldots M-1\}$ and $a_{M,M} = 1$; **X** is a column vector with $X_i = K_i$ for $i \in \{0 \ldots M-1\}$ and $X_M = V_0$; **B** is a column vector with $B_i = \langle V_n I_{n-i} \rangle$ for $i \in \{0 \ldots M-1\}$ and $B_M = \langle V_n \rangle$.

The matrix has a special form if the signal I vanishes in the last M steps, so that $\langle I_{n-i} I_{n-j} \rangle = \langle I_n I_{n+i-j} \rangle$. Then we define $a_k = \langle I_n I_{n-k} \rangle$ for all $k \in \{0 \ldots M-1\}$, and $y = \langle I_n \rangle$, so that the matrix **A** can be written as follows:

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

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 $\mathbf{Y}^T = \begin{pmatrix} y & y & \ldots & y \end{pmatrix}$.

Solving the matrix equation by block gives

$$V_0 = \langle V_n \rangle - \mathbf{Y}^T \mathbf{K} = \langle V_n \rangle - \langle I_n \rangle \sum_{p=0}^{M-1} K_p$$

$$(\tilde{\mathbf{A}} - \mathbf{Y}\mathbf{Y}^T)\mathbf{K} = \tilde{\mathbf{B}} - \langle V_n \rangle \mathbf{Y}$$

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 **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 Press et al, 1993). It is not

necessary to store all the values of V_n and I_n, since the averages $\langle V_n I_{n-j} \rangle$ can be computed online in real time (M additions at each time step).

D. EXTRACTION OF THE ELECTRODE KERNEL

To extract the electrode kernel (section 2.3), we need to solve the following equation for K_e:

$$K = K_m * \frac{K_e}{\int K_e} + K_e \tag{3}$$

where K_m is a known exponential function representing the membrane kernel (resistance R_m, time constant τ_m). We use the Z-transform to transform convolutions into products:

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

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 $K_e = K - Y$.

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FIGURE LEGENDS

FIGURE 1

Dynamic clamp with high-resistance electrodes

(a) When recording with a single electrode and injecting current at the same time, the recorded potential is $V_r=V_m+U_e$, where V_m is the membrane potential and U_e is the voltage across the electrode. Bridge compensation consists in modeling the electrode as a pure resistance: $U_e=R_e I$, where R_e is the electrode resistance and I is the injected current, and subtracting the estimated electrode voltage from the recording V_r. Because the electrode is not a pure resistance, a capacitance transient appears on the compensated trace.

(b) In dynamic clamp (here excitatory and inhibitory conductances g_e and g_i), capacitive transients are injected back and induce instabilities. If the conductances are too high or too fast (here they were low-pass filtered), the recording is unstable (no meaningful signal).

(c) The discontinuous current clamp (DCC) consists in alternating current injection and voltage recording, so that the potential is recorded at times when the electrode voltage U_e has vanished. Thus the sampling frequency is limited by the response speed of the electrode.

(d) Stable dynamic clamp is possible with DCC (here: fluctuating excitatory and inhibitory conductances), but the temporal resolution is limited: the shape of action potentials cannot be captured.

(e) Active electrode compensation is used in continuous mode, with a computer running both the dynamic clamp protocol and the electrode compensation.

(f) Stable dynamic clamp is possible with AEC with high temporal resolution.

FIGURE 2

AEC algorithm

(a) Non-Gaussian white noise current (scale bar: 0.5 nA) is injected into the neuron, as a series of independent random current steps uniformly distributed in -0.5 nA…0.5 nA. The total response V_r, corresponding to the sum of the membrane potential V_m and the voltage drop across the electrode U_e, is recorded (scale bar: 10 mV). The cross-correlation between the input current and the output voltage and the autocorrelation of the current give the kernel K (or impulse response) of the neuronal membrane + electrode system (full kernel K, right). The tail of the kernel is fit to an exponential function, which gives a first estimation of the membrane kernel Km (note: the resistance of each bin is very small since the kernel is distributed over a long duration). The electrode kernel Ke is recovered from K and Km by solving the equation K=Ke+Km*(Ke/Re) (convolution). The process is iterated several times to obtain a better estimation of the membrane kernel.

(b) A typical electrode kernel (cortical cell *in vitro*), consisting of 3 phases: 1) the first two time steps are zero, representing the feedback delay of the system; 2) a fast rise (most likely representing the electrical characteristics of the amplifier or acquisition filters); 3) a slower decay.

(c) The electrode kernel also captures all the filters on the amplifier: here, 5 kernels measured in the electronic model cell with different settings for the low-pass filter of the amplifier (.1 – 10 kHz).

(d) Once the electrode kernel has been calibrated, it is then used in real time for electrode compensation: the injected current (scale bar: 5 nA) is convolved with the electrode kernel to provide the electrode response U_e to this current. U_e is then subtracted from the total recorded voltage V_r (scale bars: 100 mV) to yield the V_m (V_{AEC}; scale bars: 10 mV, 100 ms).

FIGURE 3

Sensitivity of AEC to estimation parameters

(a) Numerical simulations show that there is a broad plateau of values of the tail parameter for which the kernel estimation is correct, as shown in this plot of estimated electrode resistance R_e vs. tail parameter (target R_e = 50 MΩ, electrode time constant τ_e = 0.2 ms, membrane time constant τ_m = 15 ms). If the parameter is too small, part of the electrode kernel is removed together with the membrane kernel and estimated R_e is too small (left). If it is too close to the total size of the full kernel (15 ms in this case), the fit of the membrane response by an exponential fails and this also leads to a wrong estimation (right).

(b) An example of a real electrode, showing that the estimated kernels are very similar for 3 different values of the tail parameter (5, 7 and 10 ms), confirming the existence of a broad plateau (regular spiking cortical cell).

(c,d) For one cortical cell recorded *in vitro*, parameters of the estimated electrode kernel for different durations of white noise (WN) injection used for the estimation (interleaved durations of 1s, 5s, 10s and 20s were used). The electrode resistance (c) is the sum of all the kernel coefficients. The electrode capacitance (d) is obtained by dividing τ by the resistance, where τ is the decay time constant of an exponential fit to the decaying part of the kernel. All the 44 kernels are shown, superimposed, as an inset in the right panel. One of the kernels (dashed line) appears as an outlier, the corresponding point on the right panel is indicated by a dashed circle. ANOVA tests showed that only the 1s WN duration produced resistance estimates significantly different from the other durations ($P<0.01$), whether or not the outlier was included in the analysis. For the capacitance, ANOVA tests showed that there was no significant difference between the different durations, or a significant difference between 1s and 10s when the outlier was removed.

FIGURE 4

Role of electrode and membrane time constants on electrode compensation

(a). We simulated a model including a passive membrane (R_m = 50 MΩ, τ_m = 20 ms) and a simple RC electrode with resistance R_e = 80 MΩ and varying time constant. Applying the AEC estimation procedure to this model shows that the quality of the electrode kernel degrades continuously when the ratio of electrode time constant and membrane time constant increases. The plot shows the estimated resistance with AEC, relative to the actual resistance (80 MΩ). The error on estimated resistance with AEC was less than 10% for τ_e/τ_m <9%.

(b) We simulated a dynamic clamp protocol with conductance noise and a slow electrode made of four resistors and capacitors (time constant 0.8 ms), impaled into a cortical cell modeled as a single-compartment Hodgkin-Huxley type model (equations and parameters in Destexhe et al,

1998), and connected to a model amplifier (emulating DCC, bridge compensation and an acquisition board; we used a model of the electrode previously published in a conference proceedings - Brette et al, 2007). The electrode time constant is much slower than our typical measurements *in vitro* (about 0.1 ms with sharp electrodes). The electrode kernel measured by AEC underestimates the total resistance (49.4 MΩ instead of 55 MΩ).

(c) With AEC, the subthreshold response is good and spikes are recorded, but in a filtered version (which is to be expected since AEC only compensate for the bias induced by injected currents).

(d) With DCC, no meaningful signal can be recorded (DCC 1: optimal setting of the sampling frequency; DCC 2: higher setting). Increasing the DCC frequency leads to unstable oscillations like in bridge (not shown). Thus, dynamic clamp recordings are possible with AEC when the electrode time constant is large and no other technique can be used.

FIGURE 5

Impact of dendrites on electrode compensation

We simulated a morphologically reconstructed layer VI pyramidal cell (Contreras et al, 1997) using Neuron, with passive properties (b-g) as in Destexhe and Pare, 1999), compatible with whole-cell patch recordings: leak conductance g_l = 0.0155 mS/cm² (range tested: 0.015-0.03 mS/cm²), resting potential V_{rest} = -80 mV, intracellular axial resistivity R_a = 70 Ω.cm (Stuart and Spruston (1998); range tested: 65-280 Ω.cm), specific membrane capacitance c_m = 1 µF/cm². In the simulations of panel e, voltage-dependent Na+ and K+ currents were inserted in soma, dendrites and axon (parameters in Destexhe and Pare, 1999). A high-resistance electrode, modeled as a resistor and a capacitor, was located into the soma (Re = 80 MΩ and τ_e = .1 ms). An additional leak conductance of 10 nS was inserted in soma to model the impalement. White noise and colored noise currents were injected through the electrode and AEC was used to correct the recording. AEC and subsequent analysis were done offline using custom Python code (http://www.di.ens.fr/~brette/HRCORTEX/AEC/) and the Brian simulator (http://brian.di.ens.fr/). Recordings were sampled at 10 kHz (Neuron simulations use a 0.01 ms integration time step).

(a) Dendritic tree of the simulated neuron, reconstructed from a layer VI pyramidal cell (Contreras et al, 1997).

(b) The kernel of the cell, as measured by direct somatic injection (solid line) is not a pure exponential function. Fitting to a sum of three exponential functions shows however that the slower one accounts for 56 MΩ of the total resistance R = 57.8 MΩ. The electrode kernel (dashed line) is concentrated on the first ms and is two orders of magnitude larger than the membrane kernel on that time scale (note the change of scale in the upper part of the figure). The neuron and electrode kernels were calculated separately, i.e., the neuron kernel was estimated with no electrode resistance, and the electrode kernel was calculated without the neuron (therefore, it is a single exponential function with time constant .1 ms); thus, these calculations did not rely on AEC.

(c) The electrode kernel measured by AEC (solid line) was very close to the real one (dashed line); the estimation gave Re = 81 MΩ instead of 80 MΩ.

(d) White noise (1st half) and colored noise (2nd half; time constant 5 ms) currents were injected through the electrode, and the voltage compensated with AEC using the measured electrode

kernel shown in (c). The AEC compensated trace (black line, foreground) is compared to real somatic potential of the model (grey line, background).

(e) This figure shows a very challenging situation for electrode compensation: nonlinear voltage-dependent conductances in the cell, electrode kernel estimated during spiking activity, large dendritic contributions to the membrane kernel, high ratio electrode resistance / membrane resistance, short membrane time constant and white noise injection. Voltage-dependent conductances were inserted, so that the cell was able to produce action potentials. As a result, the membrane resistance (and thus the time constant) at rest was halved (and even more reduced during spiking), giving a large ratio R_e/R_m = 2.5 (even larger during spiking activity: R_e/R_m). Suprathreshold white noise was injected through the electrode, inducing spiking activity. The electrode kernel was estimated with the same suprathreshold white noise injection, i.e., there were spikes during the estimation. The estimated electrode resistance was 82.6 MΩ, which was very close from the same estimation with subthreshold white noise injection (estimated R_e = 82 MΩ). The black trace shows the result of AEC compensation during the injection (grey = real somatic potential). Even though the subthreshold activity looks filtered by AEC because the fast dendritic contribution to the membrane kernel is large (10% at rest), the technique does not act as a low-pass filter: measured action potentials look very similar to the real ones.

FIGURE 6

Electrode nonlinearities

(a) Electrode nonlinearities can be measured intracellularly by running the AEC estimation procedure with different levels of additional constant currents, and comparing the resulting electrode kernels.

(b-c) The electrode resistance R_e (sum of all values of the electrode kernel) is plotted vs. the constant injected current for a very nonlinear electrode (b) and an essentially linear electrode (c). The slope of the linear regression measures the nonlinearity. The first electrode (b) should be discarded.

(d) The voltage error resulting from electrode nonlinearities can be expressed as quadratic function of the membrane depolarization, as shown on this plot for the electrode in (c) (solid line, typical of about half the electrodes in our experiments), the electrode in (b) (mixed line) and another nonlinear electrode (dashed line).

(e) We analyzed the spikes produced by 2 cells during injection of colored conductance noise *in vitro* and 1 cell *in vivo*. Because of the dynamic clamp protocol, a very large negative current was injected during spikes. Any error ΔRe in the estimation of the electrode resistance, which could be caused by electrode nonlinearities during these large current injections, would result in an error ΔRe*I in the voltage estimation. For these cells, the voltage peak was not very variable (standard deviations 0.9 mV to 1.4 mV), and the estimated resistance errors from linear regression between the injected current and the voltage at peaks were all smaller than 1 MΩ, which indicates an absence of fast nonlinearities. Electrode resistances ranged between 63 MΩ and 68 MΩ (estimated at rest with AEC).

FIGURE 7

AEC recordings *in vitro* and *in vivo*

(a) This trace is an example of a kernel estimation *in vivo*: in this case, there were spikes occurring during the white noise current injection, however the electrode kernel was very similar to a previous one obtained in the absence of spikes (measured electrode resistances were R_e = 105 MΩ in the absence of spikes and R_e = 103 MΩ in the presence of spikes).

(b) Temporal stability of the electrode properties *in vivo* for two regular spiking cells. The kernel was estimated using 5 or 20s white noise current injections. In addition different constant current levels (DC) were injected, preventing spiking activity during the estimation.

(c) Dynamic clamp protocol with 50 nS square conductance waves *in vitro* (alternation of excitation and inhibition; scale bar: 5 ms; R_e = 71 MΩ). The recording with AEC is very close to the prediction (from a passive model).

(d) Dynamic clamp protocol with fluctuating inhibitory and excitatory conductances using AEC, *in vitro* and *in vivo*. Recordings are stable and action potentials are measured with high temporal resolution.

(e) Dynamic clamp protocol with repeated injections of frozen synaptic conductance noise using AEC, in a guinea pig visual cortical RS neuron *in vitro* (left) and in a cat visual cortical RS neuron *in vivo* (right). The injected inhibitory and excitatory conductances, $G_{inhibition}$ and $G_{excitation}$, identical for each trial, are shown at the bottom. The injected current, $I_{injected}$, is displayed on top of the conductances, for only one trial; the corresponding response of the cell, V_m is displayed directly on top of the current. Subsequent V_m responses to the same conductance pattern are displayed above. Conductance noise parameters: *in vitro*, g_{e0} = 15 nS, g_{i0} = 50 nS, $s.d._e$ = 8 nS, $s.d._i$ = 6 nS; *in vivo*, g_{e0} = 12 nS, g_{i0} = 57 nS, $s.d._e$ = 3 nS, $s.d._i$ = 7 nS.

FIGURE 8

Electrode kernels resulting from typical errors (Appendix A)

(a) Appearance of the estimated electrode kernel if bridge compensation was erroneously on during white noise injection.

(b) If the size of the raw kernel is too big, the real-time estimation procedure might not follow. Here, an example of raw kernel obtained in this case, with noise dominating the kernel after 20 ms. Note that this error might manifest itself differently depending on the real-time system used.

(c) If the chosen tail parameter is too small, the electrode kernel might not contain the whole electrode response, which can manifest itself as the kernel not converging to 0 (dashed line) at its last point. In this case, the correct kernel is shown as a thick line (note that the electrode has an exceptionally slow component in this case).

(d) Ringing due to capacitance over-compensation is readily seen as a damped oscillation at the level of the estimated kernel (left). While reducing capacitance neutralization, it is important to carefully monitor that no residual oscillation remains in the kernel, i.e. that the kernel always remains positive, as is the case for the kernel shown with the thick line (right), but not for the two kernels shown with thin lines.

(e) Manifestations of fast non-linearities in the recording circuitry, when using an electronic model cell+electrode. During conductance pulse injection in dynamic-clamp, artifacts (arrows) can be seen during fast current transients (top panel). Under these recording conditions, electrode kernels obtained using white noise injections of different amplitude (bottom panel; solid line: 0.5 nA; dashed line: 1.5 nA) are not identical (arrows).

(f) After replacing one element in the recording circuitry, there are no artifacts left during conductance pulse injection. Under these recording conditions, electrode kernels obtained during white noise injections of different amplitude (as in e) are identical (perfectly superimposed on the graph). (Scale bars for e and f, top panels: vertical 5 mV, 100 nS, 2 nA, horizontal 200 ms)