Introduction

The acute *in vitro* preparation is widely used to study the cellular and synaptic properties of many brain areas. Unfortunately, this research method suffers from several unavoidable shortcomings that make it differ significantly from the *in vivo* preparation.

- First, the ionic and pharmacological environments are standardized and do not include most of the neurochemical substances present *in vivo* (ACh, DA, NE, 5HT…). The ionic environment is quickly washed out by the continuous flow of A.C.S.F., and local increase/decrease of key ions such as K+ or Ca2+ is not permitted. These local changes in ion concentrations may have significant computational roles *in vivo* (reversal potentials, ion availability…). As a consequence, the resting membrane potential *in vitro* is lower than in vivo (by about 15mV) and the input resistance *in vitro* is significantly increased (4 to 5 fold).
- Second, because of the damage induced by the slicing procedure, the synaptic activity is greatly impoverished, and spontaneous spiking activity is greatly reduced. *In vitro*, in cortex, spontaneous activity is non-existent whereas it amounts to 1-10 Hz *in vivo* (anaesthetized), and membrane potential fluctuations are small *in vitro* (<1mV) while they can be large (>10 mV) *in vivo*.

In order to diminish these shortcomings, and to mimic *in vivo* activity better, we use the dynamic clamp technique (Sharp et al., 1993; Manor et al., 1998) to inject a compensatory, voltage dependent current that will endow the cell with several properties that are observed *in vivo*: Namely, a more depolarized resting membrane potential, a 4-fold decrease of input resistance, large synaptic-like membrane potential fluctuations (~10 mV) and spontaneous spiking. This compensatory current is based on a point-conductance model of synaptic activity in which amplitude and synchrony are explicitly formulated and manipulated explicitly.

Methods

Experiments

All experiments were carried in accordance with animal protocols approved by the N.I.H. Coronal slices of rat pre-limbic and infra limbic areas of prefrontal cortex were obtained from 2 - 4 weeks old Sprague-Dawley rats. Rats were anesthetized with metofane (Methoxyflurane, Mallinckrodt), and decapitated. Their brain were removed and cut into 350 µm thick slices using standard techniques. Whole cell patch-clamp was performed under visual control at room temperature. In some experiments, synaptic transmission was blocked by D-2-amino-5-phosphonovaleric acid (D-APV; 50 µM),6,7 -dinitroquinoxaline-2,3, dione (DNQX; 10 µM), and Biccuculine methiodide (Bicc; 20 µM). All drugs were obtained from RBI or Sigma, freshly prepared in ACSF and bath applied. Data were acquired in current clamp mode using an Axoclamp 2A amplifier (Axon Instruments).
We used regularly spiking layer 5 pyramidal cells. Interneurons were recorded from layer 5/6 and were characterized by high firing rates, no adaptation and prominent fast spike re-polarization. A total of 9 pyramidal cells and 5 interneurons were used in this study.

Data acquisition

Data were acquired using a dual computer setup. The first computer was used for standard data acquisition and stimulation. Programs were written using Labview 6.1, and data were acquired with a PCI-16-E1 data acquisition board (National Instrument). Data acquisition rate was either 10 or 20 kHz. The second computer was dedicated to dynamic clamp. Programs were written using a Labview RT 5.1 front-end, and a language C back-end. Dynamic clamp (ref) was implemented using a PCI-7030 board (National Instrument) at a rate of 1 kHz. Dynamic clamp was achieved by implementing a rapid (1 ms) acquisition/injection loop in current clamp mode.

Data Analysis

Data were analyzed offline using MATLAB (The Mathworks). Results are given as mean ± standard deviation.

Point- conductance models

A point-conductance model was generated by approximating the total synaptic current due to background activity, I_{syn} , by a sum of two conductances:

$$I_{syn} = g_e(t)(V - E_e) + g_i(t)(V - E_i)$$

where $g_e(t)$ and $g_i(t)$ are time dependent excitatory and inhibitory conductances respectively; E_e=0 mV and E_i=-75 mV are their respective reversal potentials.

Both $g_e(t)$ and $g_i(t)$ were described by a one-variable stochastic processes similar to the Ornstein-Uhlenbeck process (Uhlenbeck and Ornstein, 1930):

$$\frac{dg_e(t)}{dt} = -\frac{1}{\tau_e}[g_e(t) - G_e] + \sqrt{D_e}\chi(t)$$
$$\frac{dg_i(t)}{dt} = -\frac{1}{\tau_i}[g_i(t) - G_i] + \sqrt{D_i}\chi(t)$$

where G_e =0.012 µS and G_i =0.057 µS are average conductances, τ_e=2.7 ms and τ_i =10.5 ms are time constants, D_e= 6.598 10^{-6} µS^2/ms and D_i=8.305 10^{-6} µS^2/ms are noise "diffusion" coefficients, and X(t) is a Gaussian white noise of unit standard deviation.

The advantage of the Ornstein-Uhlenbeck process is that the distribution of the stochastic variables (g_e and g_i here), and its spectral characteristics, are known analytically (see details in (Gillespie, 1996)).
The variance is given by:

$$\sigma_j^2 = D_j\tau_j / 2$$

where j stands for either e or i. The power spectral density is given by:

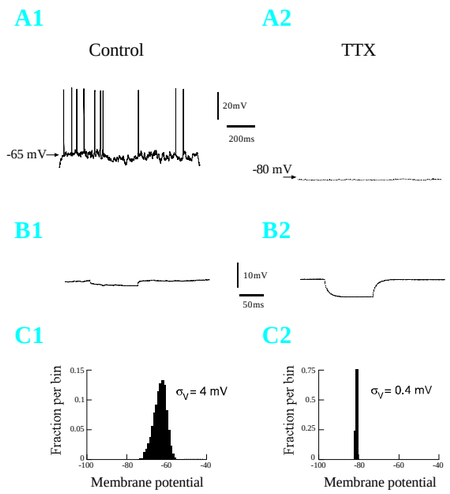
$$S_i(f) = \frac{2D_i\tau_j^2}{1 + (2\Pi f\tau_j)}$$

The Gaussian nature of the Ornstein-Uhlenbeck process, and its spectrum in 1/f, approximately match the behavior of the conductances underlying background activity in the detailed biophysical model. Using this procedure we have fit the point-conductance model to the background activity generated by a detailed biophysical model (see details in companion posters, *Destexhe and Rudolph*, panel **H-6**).

Results

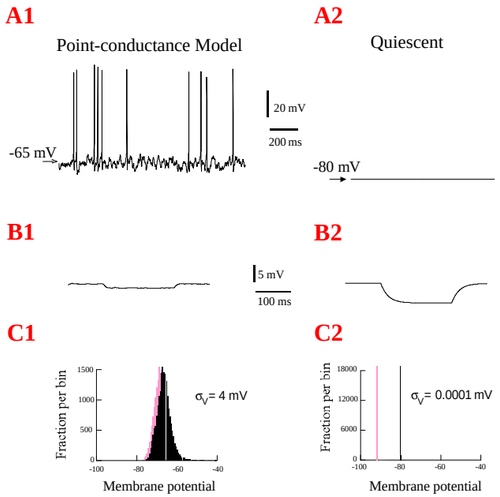
1

In Vivo



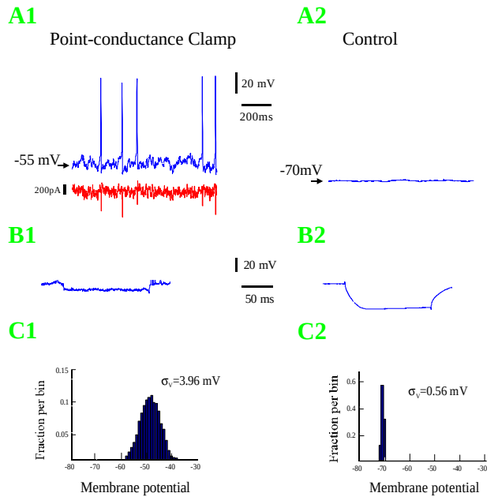
Membrane properties of a neocortical neuron in cat parietal cortex (area 5-7) during a phase of desynchronized EEG activity (ketamine-xylazine anesthesia). Figure modified from (Destexhe and Pare, 1999).
A. Intracellular recording is shown before (A1) and after (A2) microperfusion of TTX, abolishing all spontaneous activity.
B. Average of 50 hyperpolarizing pulses (-0.1 nA) to estimate the input resistance, during active periods (B1) and after TTX (B2). TTX abolished most membrane potential (Vm) fluctuations, increased the R_{in} by about 5-fold, and hyperpolarized the cell by about 15 mV.
C. Distribution of membrane potential before (C1) and after TTX (C2). TTX abolished most membrane potential (Vm) fluctuations, increased the R_{in} by about 5-fold, and hyperpolarized the cell by about 15 mV.

In Computo



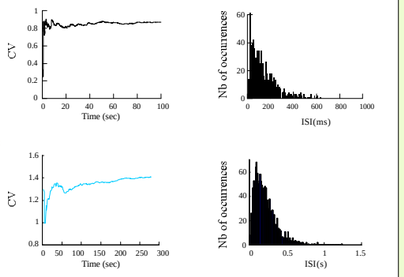
Membrane properties of the point-conductance model.
A. Time course of the membrane potential during background activity (A1) and without any background activity (A2). The membrane fluctuated around -65 mV in the active case, and around –80 mV in the quiescent case.
B. Average of 100 hyperpolarizing pulses (-0.1 nA) in the quiescent (B2) and active state (B1) when the point conductance model was introduced (G_e=0.0121 µS, G_i=0.0573 µS, σ_e=0.006 µS^2/ms, σ_i=0.0132 µS^2/ms)
C. Histograms of Vm values for a total of 5000 ms in the quiescent (C2) and active (C1) cases. The light pink histograms show the same simulation in the presence of a DC current of -0.2 nA. A significant deflection of the Vm in the quiescent case but not in the active case shows that the R_{in} was greatly reduced when synaptic background activity was introduced.

In Vitro



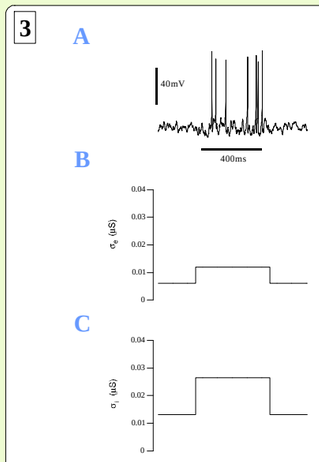
Membrane properties of the point-conductance clamp.
A. Intracellular recording of a prefrontal cortex layer V pyramidal cell in control condition (A2), and injected with the point-conductance model (A1, G_e=0.05 µS, G_i=0.05 µS, σ_e=0.024 µS^2/ms, σ_i=0.06 µS^2/ms). The point-conductance clamp depolarized the cell by about 15mV, and introduced membrane potential fluctuations (amplitude about 10 mV).
B. Average response of a different cell to a 200 pA hyperpolarization pulse in control (B2) and in point-conductance clamp (B1) conditions (7 trials, actions potentials have been truncated). The input resistance has been decreased by 4.2 fold.
C. Distribution of membrane potential before (C2) and after (C1) the activation of the point-conductance clamp. The standard deviation of the membrane fluctuations has been increased to about 4 mV.

2



Coefficient of Variation for the point-conductance model (**A,** G_e=0.05 µS, G_i=0.06 µS, σ_e=0.013 µS^2/ms σ_i=0.026 µS^2/ms) and for the point-conductance clamp (**B**) obtained from a pyramidal cell. The left panels show the evolution of CV in time, the right panels show the exponential distribution of ISIs.

3



Detection of correlation changes in the model.
Spike discharges (**A**) resulting from a change in σe and σi (shown in **B** and **C** respectively). The model detected this change in the fluctuation level, although there was no major effect on the voltage. The same behavior was also present in the detailed biophysical model following changes in correlation (see companion posters).

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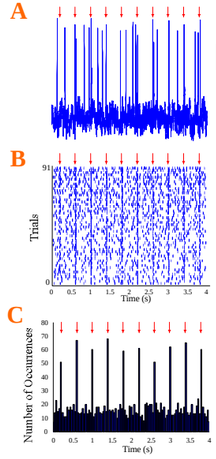
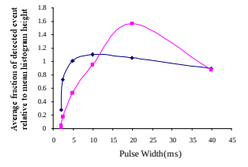


Effects of a 20 ms transient change in correlation. Each trial consisted in ten 20 ms increases of the excitatory correlation σ_e (red arrows in A, B and C), keeping the ratio σ_e/σ_i constant (0.4). The increase was two-fold (base value 0.06 µS^2/ms). The cell detected the repetitive but transient changes in excitatory correlations. The average firing rate of the cell was 6 Hz and CV was 1.37.
A. Sample trace (pyramidal cell). Note that the average membrane potential is unchanged by the transient correlation change.
B. Rastergram of 91 trials.
C. Peristimulus time histogram computed with bins of 40 ms.

5



Effect of varying the length of the transient change in correlation.
The average fraction of spike detected is plotted relative to the mean of the peristimulus time histogram for different pulse widths. The red curve was obtained for lower sigmas (σ_e=0.03 µS^2/ms, σ_i=0.075 µS^2/ms) than for the blue curve (σ_e=0.04, σ_i=0.1) in the same cell (G_e=G_i=0.05 µS). In this analysis, histograms were computed with a 10 ms time bin.

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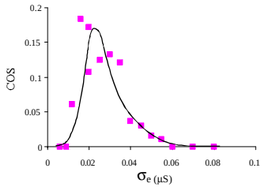

Effect of variation of the correlation parameters for transient changes of fixed length (20 ms) and fixed period (400 ms). Each point represents the COS measure computed as N_{ISI}/N_{spikes}, where N_{ISI} is the number of ISI of length equal to the period of the input (± 20 ms), and N_{spikes} is the total number of spikes across all trials. The number of trials per point varied from 6 to 11. The ratio σ_e/σ_i was kept constant at 0.4. Note that optimal detection was achieved for a restricted level of synaptic background activity. See also the *Rudolph and Destexhe* poster, panel **H-9.**

Conclusions

We showed that the combination of the dynamic clamp technique and the use of a simple point-conductance model of synaptic background activity could be used *in vitro* to mimic important properties of neurons recorded *in vivo*. These properties include:

- Spontaneous firing.

- An elevated resting membrane potential (5 to 15 mV compared to *in vitro*).

- A 4 to 5 fold decrease in input resistance (compared to *in vitro* value).

- Large membrane potential fluctuations (10 to 15 mV).

We also showed *in vitro* and *in computo* that cells placed in these conditions were able to detect transient changes in synaptic input correlations, as short as 5 ms, and that this detection relied on an 'optimal' level of background noise. *In vivo* assessment remains to be done.

We believe this technique can be a useful addition to most *in vitro* studies of neural activity and synaptic properties because it brings the *in vitro* preparation closer to the *in vivo* conditions.

References

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