Recreating active states *in vitro* with a dynamic clamp protocol

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Abstract

In neocortical neurons, network activity is responsible for an intense barrage of synaptic inputs, which maintains the membrane in a high-conductance state. Here we propose a method for recreating specific high-conductance states intracellularly. This method makes use of the estimation of the mean and variance of excitatory and inhibitory conductances based on intracellular recordings, and of the injection of appropriate stochastic conductances in *in vitro* slice preparations using the dynamic clamp protocol. The approach could be used to evaluate the modulation of neuronal responses by specific network states.

Key words: cerebral cortex, up-states, subthreshold activity, high-conductance state

1 Introduction

Cortical neurons *in vivo*, as well as in some *in vitro* preparations, are subject to a massive synaptic input resulting from the activity of the surrounding, densely connected network [1,2]. This background activity results, at the level of a cell, in a high-conductance state, which is characterized by a depolarized and highly fluctuating membrane potential (V_m), a markedly reduced membrane time constant and, often, irregular firing (for a review, see [1]).

Recently, an approach was proposed to characterize this complex network impact on a neuron by effective stochastic processes [3]. In this "point-conductance model", the background synaptic input to the neuron is described by only two global fluctuating excitatory and inhibitory conductances, $g_e(t)$ and $g_i(t)$, respectively. The relative simplicity of this model allows to use the dynamic-

clamp protocol [4,5] to mimic the effect of background activity on a cell recorded intracellularly. Moreover, it is possible to estimate precisely, from intracellular recordings, the effective conductance parameters [6,7] needed to constrain the model in order to reproduce the effects of real, specific background activity states recorded in the cell.

In the *in vitro* experiments presented here, we evaluate the adequacy of the point-conductance model, and of the analytic approach used to estimate the parameters of background activity. The goal is to develop a tool for quantitatively recreating effects of specific background activity states on cortical cells. This could allow further studies of the computational properties of neurons, such as gain control [8], in particular high-conductance states *in vitro* and *in vivo*.

2 Methods

Intracellular recordings were performed on slices of ferret visual cortex, at physiological temperatures. These slices, placed in an appropriate medium, display recurrent waves of background activity, commonly called up-states, resembling oscillations during slow-wave sleep [9].

The point-conductance model of background activity consists of a stochastic passive membrane equation subject to two independent colored Ornstein-Uhlenbeck noise processes describing excitatory and inhibitory conductances, $g_e(t)$ and $g_i(t)$, respectively [3]. For the purpose of mimicking background activity through dynamic-clamp (Fig. 1A, B), $g_e(t)$ and $g_i(t)$ are computed in real time and combined with the actual state $V_m(t)$ of the cell. The resulting current $I = g_e(t)(E_e - V_m(t)) + g_i(t)(E_i - V_m(t))$ is then injected into the cell via the recording electrode (recording and injecting current are alternated at 2.5 kHz or more, in the "discontinuous current-clamp" mode).

The parameters describing $g_e(t)$ and $g_i(t)$ were estimated from recordings of a cell during up-states (Fig. 1C, D). To that end, we recorded a number of up-states at two levels of current and removed the action potentials occurring during those up-states to capture the subthreshold activity. We then estimated the mean and variance of the excitatory and inhibitory conductances from the mean and variance of the V_m distributions as well as the knowledge of the input resistance and resting potential of the cell [7]. One crucial parameter affecting the estimates is the effective membrane area of the cell, which is difficult to measure. Therefore, we computed the conductance parameters for a range of area values (10,000 to 100,000 μm^2), and evaluated the different estimates during the reinjection of background activity.

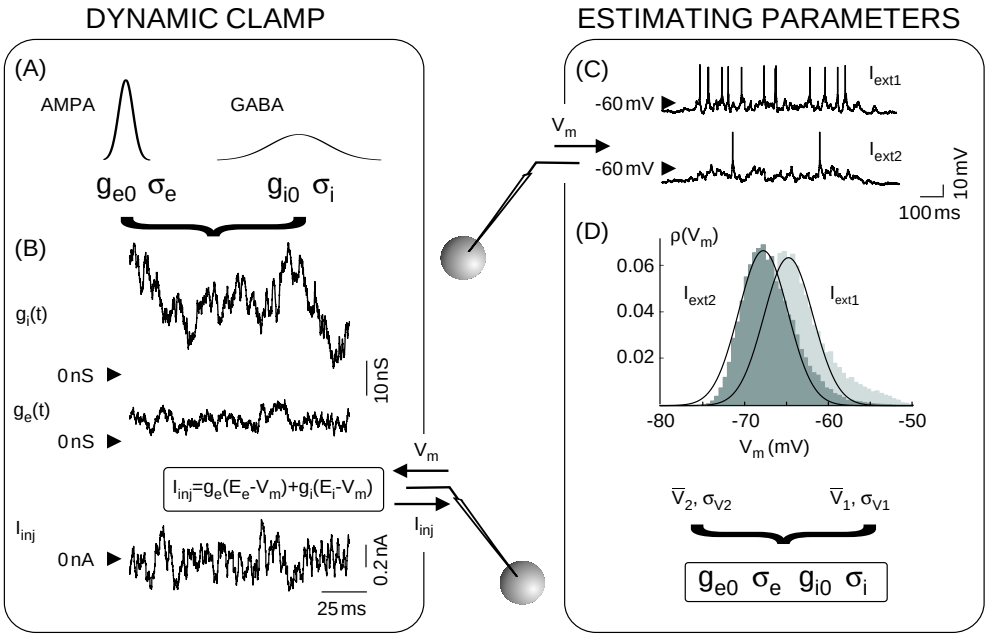

Fig. 1. Experimental methods. A,B: Reproducing background activity with dynamic clamp. Background activity is modelled with two effective conductances (A), $g_e(t)$ and $g_i(t)$, following Gaussian distributions of specified parameters (g_{e0}, g_{i0}, σ_e, σ_i). The current (I_{inj}) resulting from the activation of those conductances is computed in real time and takes into account the V_m of the cell at each time step (B). Recording and injection are alternating through the recording electrode (right). C,D: Estimating parameters of background activity conductances from intracellular recordings. The V_m of the cell is recorded at two levels of current I_{ext1} and I_{ext2} (C) and V_m distributions are obtained after removal of spikes. The mean and standard deviation of the V_m distributions are used to estimate the mean (g_{e0}, g_{i0}) and standard deviation (σ_e, σ_i) of the excitatory and inhibitory conductance distributions (D).

3 Results

We performed two sets of experiments. The first set of experiments was aimed at testing the impact of intrinsic cell properties not taken into account by our model. We injected background activity with known parameters in a cell, using the dynamic-clamp method. We then tried to retrieve these parameters from the recorded V_m, using the analytic approach. In this case, we controlled perfectly spatial effects (injection and recording at the same location, through the same electrode) and conductance distributions (imposed with dynamic-clamp). Discrepancies between parameters chosen and parameters retrieved from recordings would have indicated that V_m dynamics were not properly described by the passive membrane equation. However, in the real cell studied here, we could effectively retrieve the parameters with the analytic method (Fig. 2).

In the second set of experiments, we proceeded to recreate in neurons high-

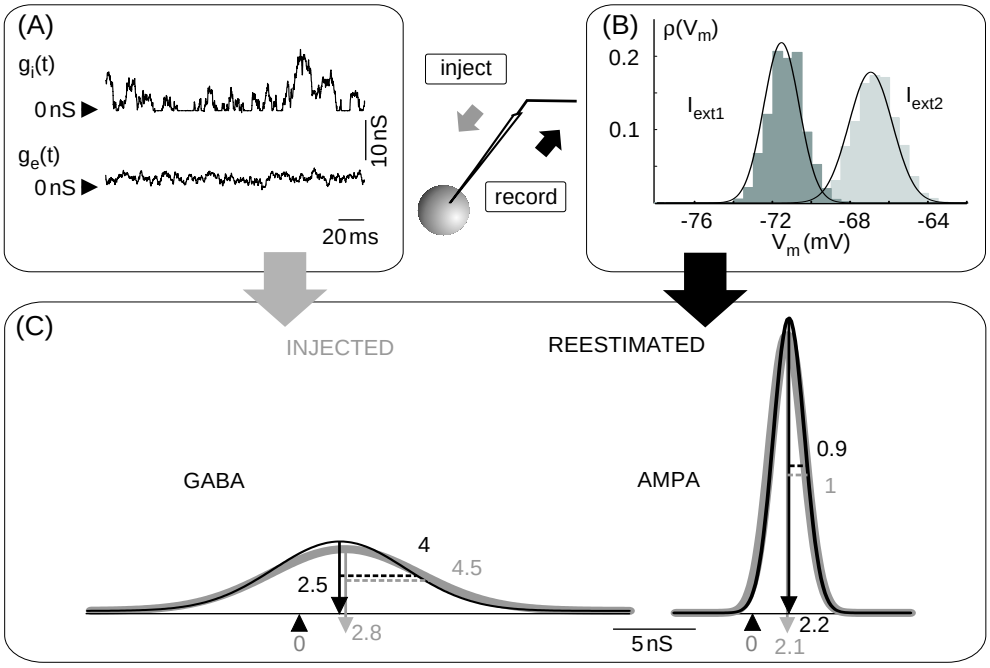

Fig. 2. Retrieving known conductance parameters from intracellular recordings. A: Two fluctuating conductances $g_i(t)$ and $g_e(t)$ with given parameters are generated in real time and injected into the cell with dynamic-clamp. B: The V_m of the cell is recorded at two levels of current I_{ext1} and I_{ext2} and V_m distributions are obtained. C: The mean and standard deviation of the V_m distributions are used to estimate the mean (arrows) and standard deviation (dashed lines) of the inhibitory (GABA) and excitatory (AMPA) conductance distributions. The estimates (black) are compared with the known parameters (grey) and match very well.

conductance states resulting from activity generated by the slice and mediated by real synaptic connections. The extent to which these states could be well approximated by the stochastic process we use depends on the dynamics of the active network in the slice, on the dynamics of synapses (time course of EPSPs) and on the processing by the dendritic tree. We show here one example of a real cortical cell recorded in active slices, for which we were able to estimate the parameters of the background activity generated by the slice, and then recreate comparable states (in terms of somatic voltage distributions) in the same cell using the point-conductance model in a dynamic-clamp approach (Fig. 3).

4 Conclusions

Our experimental results provide an important confirmation for one approach of background activity that has been developed theoretically. Under the assumptions of a point-conductance model (passive membrane dynamics subject

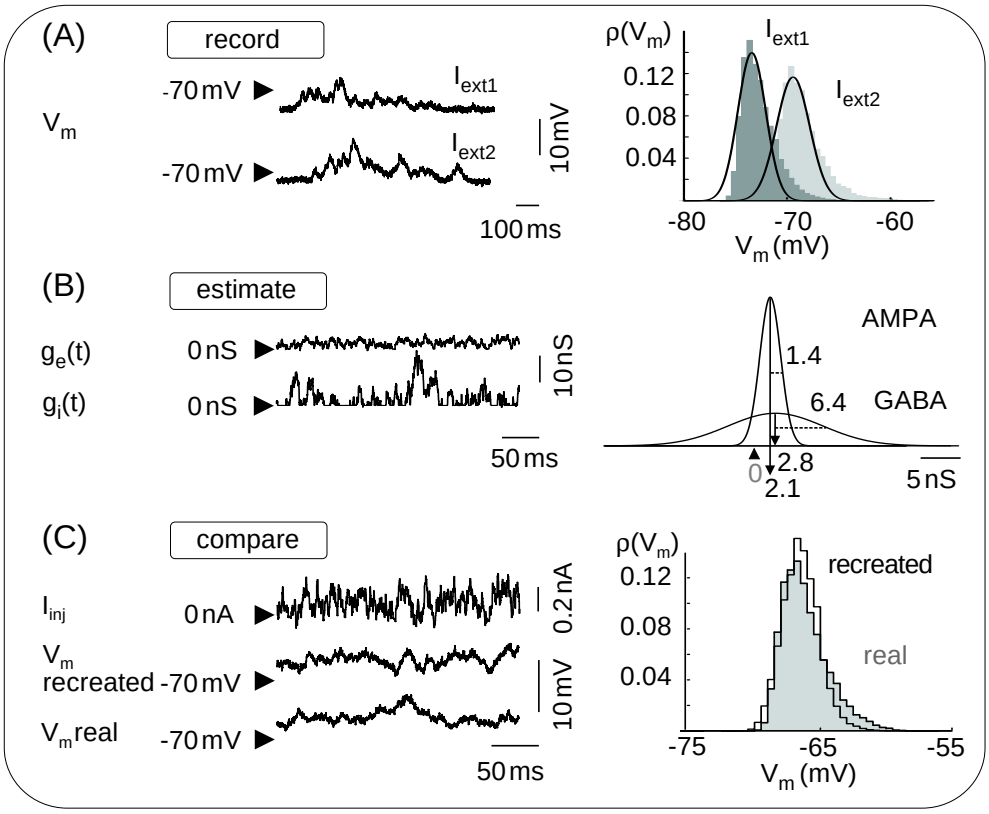

Fig. 3. Recreating high-conductance states in a neuron from intracellular recordings of background activity generated by the slice. A: Upstates are recorded at two levels of constant stimulating current I_{ext1} and I_{ext2} (left). V_m distributions are obtained from those upstates (right). B: The mean and standard deviation of the V_m distributions are used to obtain the mean (arrows) and standard deviation (dashed lines) of the excitatory (AMPA) and inhibitory (GABA) conductance distributions. Those distributions are used to generate in real time the fluctuating conductances $g_e(t)$ and $g_i(t)$ (left). C: Fluctuating conductances are injected into the cell with dynamic-clamp (I_{inj}, total injected current). The resulting V_m state of the cell (V_m recreated) is compared to the V_m during upstates (V_m real). V_m distributions in those two cases match very well (right).

to two stochastic synaptic conductances), parameters of the background activity can be extracted from intracellular recordings; those parameters, used under the same assumptions in a dynamic-clamp protocol that allows to inject conductance in the recorded cell, let us recreate states quantitatively similar to the states recorded during real network activity. This result, in keeping with prior modelling studies [6,7], points to the fact that the model and its parameters capture some essential information about network activity in the cortical active slice.

Furthermore, the technical possibility, demonstrated here, of precisely recreating specific active states at the level of a neuron, opens the way for future in-depth studies of the effects of well-controlled background activity on phe-

nomena such as synaptic integration and plasticity. The somatic injection of conductance in the dynamic-clamp protocol is a limitation that might prove important for such studies. However, future patch-clamp experiments using similar protocols as those described here might partly overcome it by giving better control of the dendritic voltage. Ultimately, the point-conductance approach should be tested in similar experiments *in vivo*. It could prove a useful tool in investigating whether small adjustments in some parameters of the so-called background activity could be used by the brain for fine-tuning information processing at the levels of neurons and networks.

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