Estimation of synaptic conductances and their variances from intracellular recordings of neocortical neurons *in vivo*

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Abstract

During intense network activity, neocortical neurons are in a "high-conductance" state. To estimate the respective contributions of excitatory and inhibitory conductances in generating such states, we combined computational models with intracellular recordings obtained in cat parietal cortex *in vivo*. Fitting a fluctuating-conductance model to the recordings revealed that inhibitory conductances are dominant (several times larger than excitation). Conductance variance (i.e., the "noise") was also larger for inhibition, indicating that inhibitory dynamics has a pronounced impact on membrane potential fluctuations. We conclude that the synaptic bombardment of neocortical neurons *in vivo* is not excitatory, but mostly determined by inhibitory conductances.

Key words: cerebral cortex, synaptic noise, subthreshold activity, stochastic systems

1 Introduction

In the neocortex, pyramidal cells are embedded in a very dense network, each receiving many thousand synaptic inputs from other neurons. In cat parietal cortex, neurons fire spontaneously at relatively high rates (1-20 Hz on average) in awake animals [1]. Therefore, these cells are subject to a sustained synaptic bombardment, which results in a high-conductance state characterized by a highly fluctuating intracellular activity [2]. Here, we combine computational

models and analytic methods with intracellular recordings of morphologically-identified pyramidal neurons in cat parietal cortex to estimate the conductance due to synaptic background activity *in vivo*. In particular, we focused on evaluating the respective contributions of excitatory and inhibitory conductances in setting the dynamics of high-conductance states.

2 Methods

Cortical neurons were recorded intracellularly in area 5-7 of cats anesthetized with ketamine-xylazine. Under this anesthesia, cortical neurons display recurrent periods of activity ("up-states") similar to the wake state. In some experiments, stimulating electrodes were placed in the pedonculopontine tegmental (PPT) nucleus. PPT stimulation evoked periods of low-amplitude fast-frequency EEG activity ("post-PPT states") lasting up to 20-30 sec, during which the input resistance (R_{in}) and membrane potential (V_m) fluctuations were measured.

These *in vitro* intracellular measurements were used to directly constrain detailed biophysical models of morphologically-identified neocortical pyramidal neurons in which synaptic background activity was simulated by random release events at glutamatergic and GABAergic synapses [3]. Analytic methods based on the statistical characterization of intracellular activity were used to estimate the mean and variance of synaptic conductances [4,5].

3 Results

Under ketamine-xylazine anesthesia, recurrent periods of activity are generated which are comparable to the wake state: neurons fire tonically at 1-20 Hz, the membrane potential is depolarized by several mV with respect to the resting state and displays large amplitude fluctuations (Fig. 1A, up-states; Fig. 2A, left for sample trace), and the electroencephalogram (EEG) is characterized by low-amplitude fast activity [6,1]. These periods of intense activity will be referred to as "active periods". Similar active periods were obtained when stimulating electrodes were placed in the pedonculopontine tegmental (PPT) nucleus. The PPT evoked active periods lasted 20 to 30 s and were were also always paralleled with low-amplitude fast-frequency EEG, and displayed similar electrophysiological characteristics (Fig. 1A, post-PPT; Fig. 2A, right for sample trace) as neurons recorded in awake animals [1,7] (for a review see [2]).

In previous work [8,9], we measured the effect of network activity on the input resistance (R_{in}) by comparing intracellularly-recorded cells in active periods

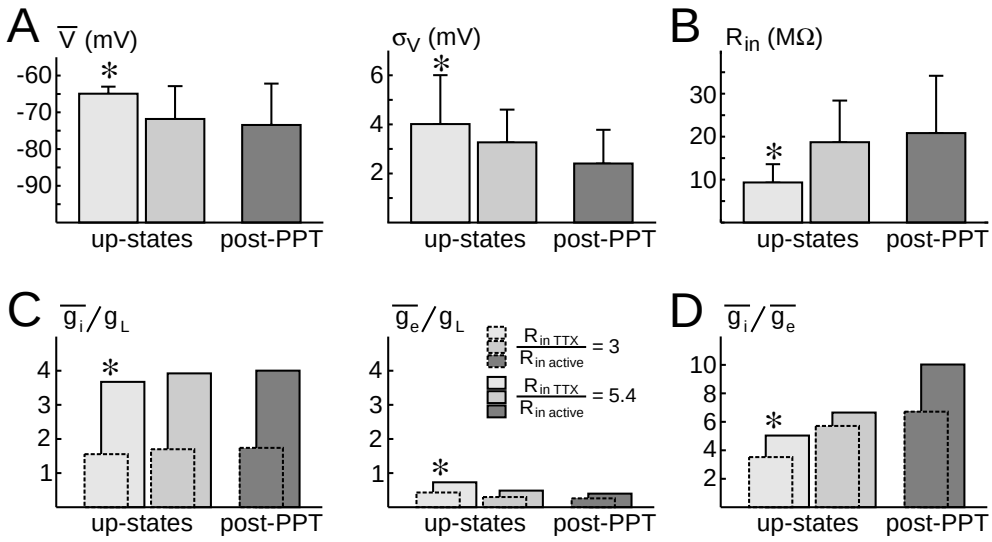

Fig. 1. Characterization of intracellular activity and the contribution of excitatory and inhibitory conductances during "active periods" under ketamine-xylazine anesthesia. Up-states and post-PPT are characterized by a marked depolarization and large membrane potential fluctuation amplitude compared to rest (**A**), as well as low input resistance (**B**). Estimates of the ratio between mean inhibitory and leak conductance (**C**, left) as well as mean excitatory and leak conductance (**C**, right) were obtained by incorporating measurements of the average membrane potential into the passive membrane equation (Eq. 1). These estimates yield a several-fold larger mean for inhibition than excitation (**D**). Results for two ratios between R_{in} under TTX R_{inTTX} and in active states $R_{inactive}$ are shown (**C** and **D**, dashed and solid boxes). The stars indicated previously obtained results [8,9].

and after microperfusion of tetrodotoxin (TTX) to the cortex. This analysis revealed that active states are characterized by a dramatically larger (about 500%) total conductance (5 times smaller R_{in}) compared to the resting state obtained after TTX. Synaptic activity was also responsible for an important depolarization (average V_m of $\overline{V} = -65$ mV $\pm$ 2 mV, compared to -80 mV after TTX) and large amplitude V_m fluctuations (V_m standard deviation of σ_V=4 $\pm$2 mV). We report here similar values during active states following PPT stimulation (Fig. 1B. However, the R_{in} tended to be higher (up to 40% difference) and the V_m more hyperpolarized (a few millivolts) than during ketamine-xylazine anesthesia. Because the PPT participates to the ascending activating system during wakefulness, these states are electrophysiologically close to aroused states.

To estimate the respective contribution of excitatory and inhibitory conductances, we integrated these measurements into the passive membrane equation:

$$C_m \frac{dV_m}{dt} = -g_L(V_m - E_L) - \overline{g}_e(V_m - E_e) - \overline{g}_i(V_m - E_i)\,, \qquad (1)$$

where g_L and E_L denote the leak conductance and reversal potential, $\overline{g}_e$, $\overline{g}_i$,

E_e and E_i denote the mean excitatory and inhibitory conductance and their reversal potentials, respectively. We obtain estimates of $\overline{g}_e/g_L = 0.49$, $\overline{g}_i/g_L = 3.91$ for up-states and $\overline{g}_e/g_L = 0.40$, $\overline{g}_i/g_L = 4.0$ for post-PPT states (Fig. 1C). The resulting ratio $\overline{g}_i/\overline{g}_e$ is therefore about of 6.6 on average for up-states under ketamine-xylazine, and 10.0 for active periods after PPT stimulation (Fig. 1D).

To check for consistency, we used the above conductance values in the passive equation to predict the average V_m in conditions of reversed inhibition (chloride-filled pipettes; measured E_{inh} of -55 mV). The predicted $\overline{V}$ was of -51.9 mV, which is remarkably close to the measured value of $\overline{V} = -51$ mV [8,9]. This analysis therefore shows that for all experimental conditions (ketamine-xylazine anesthesia, PPT-induced active states, and reversed inhibition experiments), inhibitory conductances are several-fold larger than excitatory conductances. This conclusion is also in agreement with the strong inhibitory conductances measured in voltage-clamped cortical neurons during visual responses *in vivo* [10].

To determine the absolute values of conductances and their variance, we used a method consisting in fitting the experimental V_m distributions with the analytic solution obtained from the above membrane equation when g_e and g_i are described by random walk processes [11]:

$$\frac{dg_u}{dt} = -(g_u - g_{u0})/\tau_u + \sqrt{D_u}\,\xi_u(t)\,, \qquad (2)$$

where g_{u0} denotes the mean (static) and $2\sigma_u = \sqrt{D_u\,\tau_u}$ the standard deviations for excitatory and inhibitory synaptic conductances ($u = \{e, i\}$; ξ_u are independent Gaussian white noise processes, D_u the noise diffusion coefficients and τ_u the noise time constants). The explicit form of the membrane potential probability distribution at steady-state, which can be deduced by solving the stochastic membrane equation [4], yields analytic expressions for the mean $\overline{g}_u$ and standard deviations σ_u of the synaptic conductances as functions of the mean and standard deviation of the membrane potential distribution at two different injected constant currents (see details of the method as well as *in vitro* applications in [5]).

Applying this procedure to the active states of ketamine-xylazine anesthesia led to the following values for the synaptic conductances: $g_{e0} = 57$ nS, $g_{i0} = 185$ nS (ratio $g_{i0}/g_{e0} \sim 3.52$), $\sigma_e = 9.7$ nS, $\sigma_i = 36.6$ nS (ratio $\sigma_i/\sigma_e \sim 3.77$) for up-states (Fig. 2B and C, left), $g_{e0} = 9.1$ nS, $g_{i0} = 77.3$ nS (ratio $g_{i0}/g_{e0} \sim 8.49$), $\sigma_e = 4.5$ nS, $\sigma_i = 10.4$ nS (ratio $\sigma_i/\sigma_e \sim 2.28$) for post-PPT states (Fig. 2B and C, right). These values obtained by this independent method confirm the above estimation that inhibitory conductances are several-fold larger than excitatory contributions. Inhibitory conductances also display

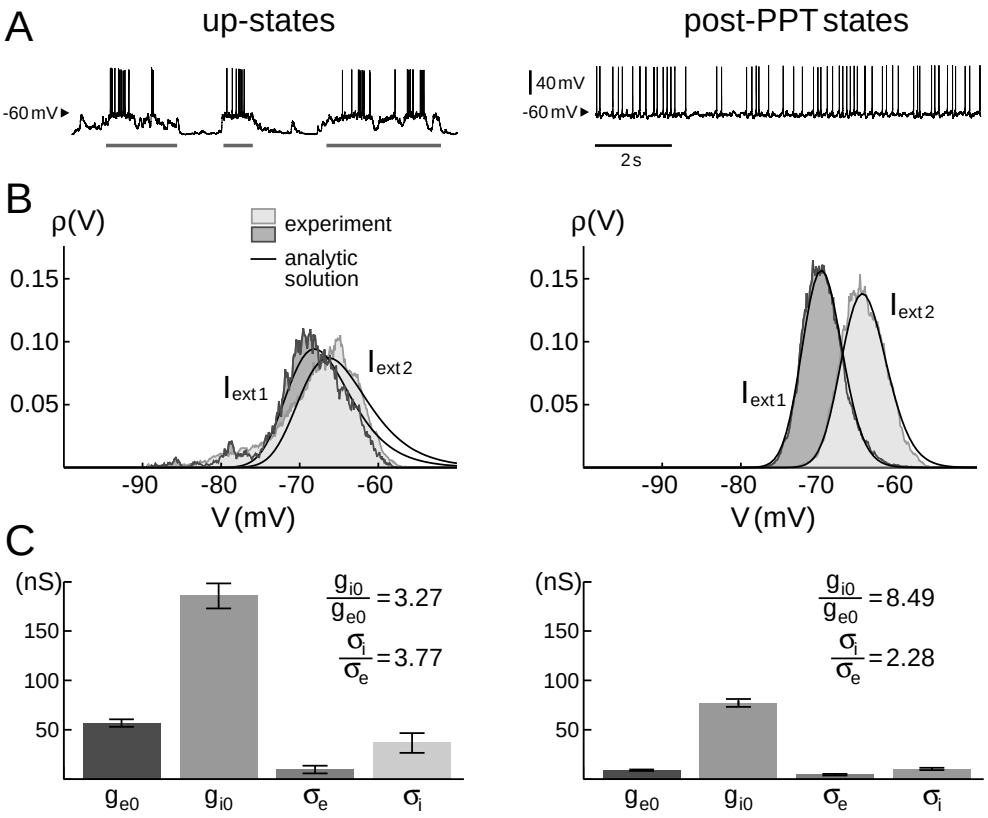

Fig. 2. Mean and variance of synaptic conductances during "active periods" under ketamine-xylazine anesthesia. **A**: Examples for intracellular activity during ketamine-xylazine anesthesia (left, up-states indicated by bars) and post-PPT states (right). The resulting membrane potential distributions $\rho(V)$ for up-states (**B**, left) and post-PPT states (**B**, right) at two different injected currents I_{ext1} and I_{ext2} can be used to estimate the mean of excitatory and inhibitory conductances (g_{e0} and g_{i0}, respectively) as well as their standard deviations (σ_e and σ_i, respectively; for details of the Method see [5]). These estimates predict a several-fold larger contribution of inhibition over excitation.

the largest variance and have a determinant influence on V_m fluctuations. These predictions should be testable by dynamic-clamp experiments (see also Destexhe *et al.*, this volume).

4 Conclusions

In summary, combining computational models and analytic methods with intracellular recordings of cortical neurons during active states *in vivo* suggest that these neurons in a high-conductance state are dominated by inhibitory conductances. This conclusion is reached by using two different methods, and is also consistent with recordings with reversed inhibition. The standard deviation of the conductance was also large for inhibition, suggesting that inhibitory

conductances provide a major contribution in setting the high levels of fluctuations characteristic of active states. We therefore conclude that the "synaptic bombardment" of neocortical neurons during active states is not excitatory, as often assumed, but is mainly determined by the dynamics of inhibitory conductances.

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