Assessing Neuronal Connectivity in Cortical Cultures from Calcium Imaging Signals

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Large networks generate complex behavior, as is apparent in diverse systems such as computers, society and biology. Particularly attractive systems are neuronal tissues, with the brain the most prominent example.

Neuronal tissues show a wiring architecture and dynamics that are still far from being completely understood. Surprisingly, when natural neuronal tissues are dissociated and left to grow on a glass cover slip, their neurons self-organize to form a new network characterized by rich spontaneous dynamics and a non-trivial connectivity. The exquisite balance between the relative simplicity of these neuronal cultures and their rich behavior have made them one of the most attractive model systems in Physics [1].

Spontaneous activity in cultures is characterized by episodes of neuronal bursting in which the network fires collectively in a short time window, combined with silent intervals of relatively low activity (Fig.1a). In our experiments we monitor the spontaneous activity of 2000-4000 neurons in rat cortical cultures using high-speed fluorescence calcium imaging, and in combination with diverse perturbations of the neuronal network.

Connectivity is modified by targeting the excitatory and inhibitory synapses. We consider for instance the blockade of inhibitory synapses with bicuculline, a $GABA_A$ antagonist, to study the influence of inhibition on dynamics. We also consider the gradual disintegration of the excitatory connectivity with CNQX, an AMPA-receptor antagonist, as described in Refs.[2,3]. As shown in Fig.1a, the fluorescence amplitude of the bursts decreases with [CNQX], while the inter-burst interval increases, indicating a reduced excitability of the neurons in the network. The normalized amplitude $(F - F_0)/F_0$, with F_0 the baseline fluorescence, rapidly decreases with [CNQX] (Fig.1b).

The detailed analysis of the changes in the fluorescence amplitude of all neurons in the network provides information on the excitability of the system, and allows the identification of special traits such as strongly connected regions. Finally, we also note that fluorescence amplitude can be related with the number of actions potentials of a neuron [4]. This fact, in combination with graph-theoretical concepts and Transfer Entropy algorithms, may provide information of additional connectivity features of the network. Those features may be crucial for a better understanding of the interplay between activity and connectivity.

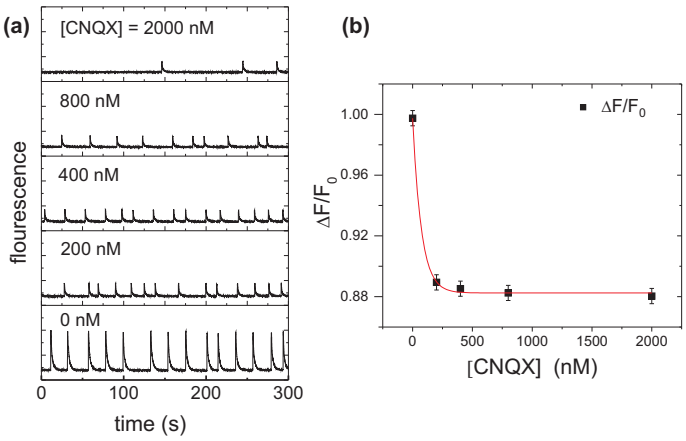

FIG. 1. **(a)** Fluorescence signal F of the spontaneous activity in a neuronal culture, averaged over 2000 neurons, for different concentrations of CNQX. **(b)** Average values of $\Delta F/F_0$ (squares) as a function of the CNQX concentration. $\Delta F = F - F_0$ and F_0 is the fluorescence baseline. The red line is a guide to the eye.

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