Assessing Connectivity in Living Neuronal Networks: Experiments and Model

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Neuronal cultures [1] provide a simple yet versatile experimental platform to monitor the behavior of a living neuronal network, and model it through Physics toolboxes such as dynamical systems or network theory. These *in vitro* networks are prepared by plating an ensemble of neurons over a substrate, which quickly connect to one another and shape within a week a *de novo* assembly with rich activity.

Here we present different studies that illustrate the potential of these cultures. Two kinds of preparations are of special interest, namely *homogeneous* (Fig. 1A-B) and *aggregated* (Fig. 1C). For the former, about 5000 neurons cover quasi–uniformly the substrate. For the latter, neurons form compact aggregates —about 50 in number— connected to one another. The easy access and manipulation of these cultures have allowed us to investigate aspects such as spontaneous activity patterns [2], connectivity[3], development [3], and the resilience of the neuronal circuit to damage [4].

Data is acquired through fluorescence calcium imaging, which allows the simultaneous monitoring of thousands of neurons in the field of view. Active neurons are visualized as bright spots on the images (Fig. 1A-B), which are processed to obtain, for each neuron, the fluorescence profile along time (Fig. 1D). The analysis of this data allows, on the one hand, the investigation of collective phenomena such as coherent dynamics (yellow box in Fig. 1D); and, on the other hand, the tracking of activity propagation by determining the time delays between neuronal activations (Fig. 1E).

Homogeneous cultures have proven invaluable to understand the mechanisms behind the initiation and propagation of spontaneous activity [2]. By resolving the ignition times of all the monitored neurons in the culture, we observed that activity propagation occurs in the form of circular waves. Activations occur quasi–periodically along time and, surprisingly, initiate in a few, well–defined locations in the culture, which we call 'nucleation points' [2]. The potential of the analysis is that the nucleation points, as well as the front propagation traits, strongly depend on the circuitry of the network. In a framework that combines dynamical systems, noise, and graph theory, we extracted interesting properties of macroscopic patterns and their link to the underlying connectivity map.

A complementary strategy consists in stimulating externally the neuronal network while the connections among neurons are progressively weakened [3]. This protocol leads to a scenario in which the largest group of connected neurons defines a *giant component* that decreases in size as the network is gradually disconnected through a control parameter m (Fig. 1F). At a critical disconnection degree m_C, the giant component disappears. The analysis of the data in the context of percolation in networks shows that the value of this critical point is tightly related with the average connectivity of the network, and that the shape of the disintegration curve depends on the details of the connectivity, such as the distribution of connections.

Finally, aggregated cultures show a radically different, modular dynamics. Their study and manipulation provide a unique platform to investigate network theory aspects in living networks [4] and resilience to damage (Fig. 1G).

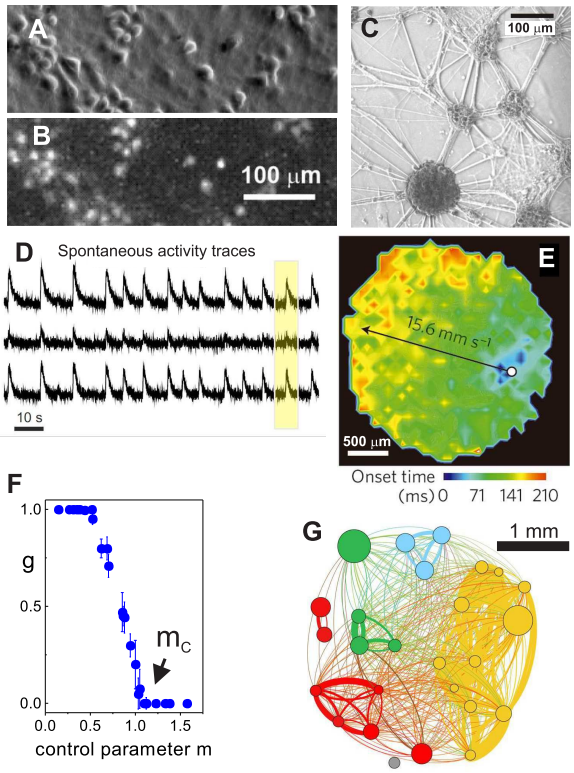

Figure 1: (A-B) Homogeneous culture, in bright field and fluorescence. White spots are active neurons. (C) Aggregated culture. (D) Spontaneous activity traces for 3 neurons. The yellow box marks a coherent event. (E) Propagation of activity in the form of a circular wave. (F) Size of the giant component as a function of the disintegration parameter m. (G) Activity propagation among aggregates. The wider the link, the fastest the flow of activity. Colors denote modules or groups of clusters that tend to fire together.

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