Self-organization and evolution of function and structure in cultured neuronal networks.

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The study of cultured neuronal networks (CNN) has recently achieved a major relevance as an alternative to *in vivo* models, being a simplified version of the central nervous system while still having a complex self-organization [1,2]. CNNs are a widely used model to analyze both the topological and functional network in the nervous system, helping us to understand the complex relationship between structure and dynamics in neuronal networks and how it changes along the development process of the culture. However this understanding is often limited by the difficulties to resolve the structure of neural networks. Our experimental approach allows us to simultaneously study the detailed structure and dynamics of the cultured network and, therefore to compare the topological and functional networks through the forming process.

We grow CNNs from isolated neurons extracted from *Schistocerca gregaria* (locust) on top of microelectrode arrays (MEA), allowing the recording of their electrophysiological signal (Fig.1) for 14 days. Neurons start to developing connections among them after 3 days in vitro (DIV). We acquire large scale microscope images of the culture from which we obtain the structural networks using a homemade image segmentation algorithm. Simultaneously, we record the electrical time series (25min) from the 120 electrode channels to detect the spikes timestamps. We analyze the spikes pair-wise synchronization events along the series using cross-correlation measures to finally produce the functional network (Fig. 1).

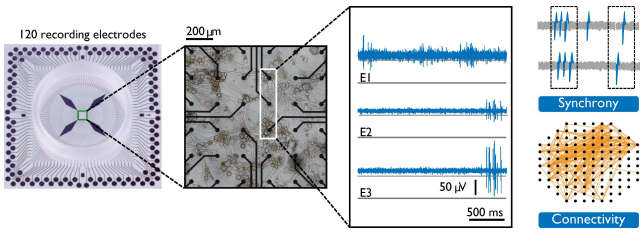

Fig. 1. Cultured Neuronal Networks are recorded on top of MEA. The signal from 120 electrodes corresponding to neuronal network activity is analyzed to study synchronization and connectivity of the functional network.

After DIV 3, the clustering coefficient in the self-organized formed structural network increases and the shortest path decreases (Fig. 2(a)), revealing small-world properties in the mature state of the network. MEA recordings of the CNN show an increase of the firing spikes activity in the active electrodes (Fig. 2(b)). As an average synchronization measure,we evaluate the mean value of the correlation matrix normalized by the number of active electrodes (Fig. 2(c)). This peak of activity and synchronization between DIV 4-6 is linked with the percolation of the topological network. A second peak, between DIV 10-12 is observed when the topological network reaches full maturity. Future work aims to a more detailed statistical analysis of the functional network and a closer comparison of the structural and functional evolution of the CNN along the developmental process.

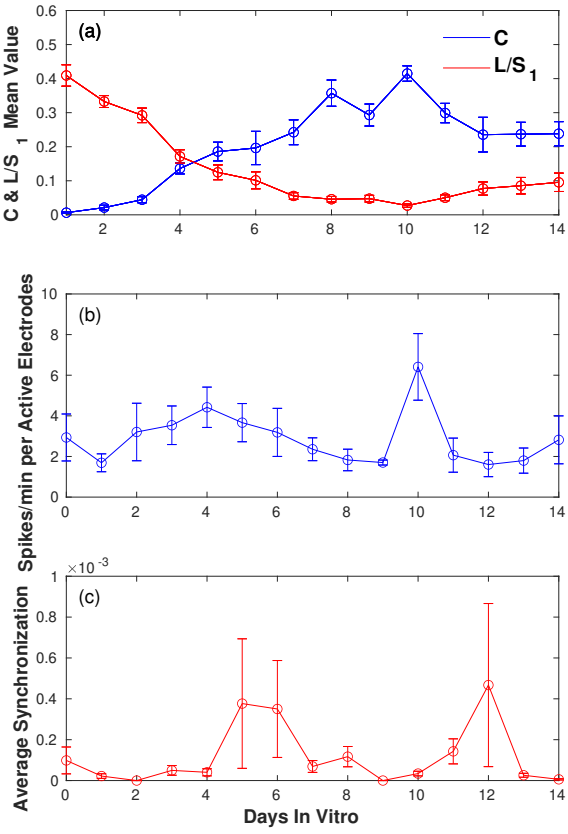

Fig. 2. (a) Clustering coefficient (C) and shortest path values (L/S_1) in the topological network analysis during 14 days in vitro. (b) Spikes/min per active electrode of MEA recordings and (c) average synchronization of the functional network.

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[2] A. Tlaie, L. M. Ballesteros-Esteban, I. Leyva, I. Sendia-Nadal, *Statistical complexity and connectivity relationship in cultured neural networks*, Chaos 119, 284 (2019).