Motifs in the Functional Connectivity of Primary Neuronal Cultures

Cultured Thoughts: (Questions on) The structure and dynamics of mammalian neuronal networks ex vivo

Work in progress and in collaboration with Guenter Gross (Texas) and Luis Bettencourt (Los Alamos)

Outline

How do we understand the complex interplay between network structure and network dynamics that provides the functional neural basis for behavior?

(e.g in vitro imaging of dendritic spines…)

I. Introduction to Cultured Networks
II. Exploration of Network Dynamics. Coordinated bursting, pharmacological control and modelling
III. Connectivity through Correlation. Analyzing the global pattern of synaptic connections
IV. Conclusions

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Spinal Cord Network Grown on 64-electrode Array

Cultured network shown 95 days after seeding. The indium-tin-oxide conductors are 10 µm wide. Horizontal electrode spacing is 40 µm, vertical spacing is 200 µm. Insets: close-ups of neurons within the network. Bars: 80 µm. Loots-modified Bodian stain.

Sugar, Spice and Everything Nice: What are Cultured Networks Made of?

1. BALB-C/ICR mice are mated for 24 hours, fourteen days before culturing.
2. For each batch of cultures, a single pregnant female mouse is anaesthetized, sacrificed by cervical dislocation, and dissected under sterile conditions to remove the uterus.
3. Ten to fourteen embryos are dissected from the uterus under a dissection microscope in sterile DMEM.
4. Each fetus is decribrated and has its spinal cord or frontal cortex (at red line) removed. The dorsal root ganglia and meninges are removed from the spinal cord, and only the strict portion of the spinal cord is used.
5. The D15GH is aspirated and the tissue is minced with two sterile #20 scalpels.
6. The spinal cord pellet is triturated in 5 ml MEM + 10% horse serum/10% fetal bovine serum (MEM 10/10). Minced frontal cortex is triturated in 5 ml DMEM + 5% horse serum/5% fetal bovine serum + B27 + 10 µg vitamin C (DMEM 5/5).
Culture Preparation Advantages

- Hundreds to thousands of neurons. Substantial (possibly complete) sampling of 2D mesoscopic network.
- Dynamical timescales ranging from milliseconds to months with 2-3 weeks in *ex vivo* development.
- Electrodes can stimulate and record. Learning and adaptation (Eytan et. al. 2003).
- Simple pharmacological manipulation of network activity.

Culture Dynamics

(to what class of dynamical systems do cultured networks belong?)

Network bursts are easily recognizable dynamical patterns. Others are certainly possible (e.g. synfire chains).

**Bursting Networks as a Self-Organized Critical System**
(organotypic cultures, Beggs et. al. 2003)
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The Culture Clock:
An NMDA-only mediated excitatory network

NMDA-only Network Rate
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**NMDA-only Burst Statistics**

What controls the onset, duration and magnitude of these quasi-regular bursts?

\[
I_{ji}^{sym}(t) = \sum_j g_{ji}^{max} s_{ji}(t) (V_i - V_{ji}^{rev})
\]

\[
g_{ji}^{max} = \frac{g_{ji}}{1 + \frac{[Mg^{2+}]exp}{3.57mM} \frac{V_i - V_L}{10^{-13}mV}}
\]

**Connectivity through Correlation**

The complex computational processes implicit in even simple behavioral tasks rely on the ability of elaborate neuronal networks to code, process and store information.

Unfortunately, current understanding of even relatively small networks is limited and a number of important questions remain open or only partially answered.

- What are the rules by which a complex neural circuit develops?
- How are the resulting networks classified (are they random or structured)?
- How does ongoing neural activity sculpt the network (plasticity and learning)?
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Synaptic Correlations in Spinal Cord Cultures

The Cross-Covariance Function

\[ \hat{\epsilon}_{12}(j) = \sum_{i=1}^{N} s_1(i) \cdot s_2(i+j) - < s_1 > < s_2 > \]

Common Input and Data Corruption

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The Correlation Zoo

Correlation Peak Clustering

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Beyond Cross-Correlation
(rephrase functional connectivity using information theory, London et. al. 2002)

From Local to Global
(the small world of cultures?)

Cultured Network

Random Network
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Conclusions

- Neurons cultured on multi-electrode arrays provide a unique and powerful tool for understanding the interplay between the structure and dynamics of complex neuronal networks.

- As a compromise between the simplicity of a single neuron and the daunting complexity of an intact neural system, cultured networks offer an opportunity to productively combine physiological and computational approaches.
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The Art of Culture