Development of Input Connections in Neural Cultures

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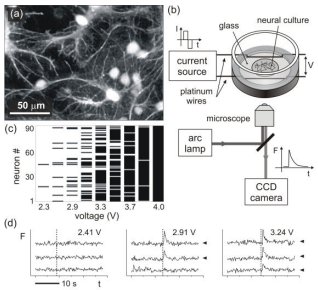
Abstract

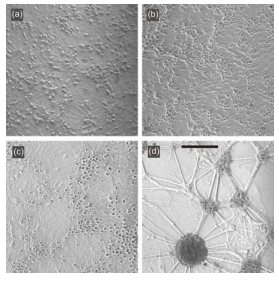
We introduce a novel approach for the quantitative assessment of the connectivity in neuronal cultures, based on the statistical mechanics of percolation on a graph. This allows us to follow the development of the culture and see the emergence of connectivity in the network. The culture becomes fully connected at a time equivalent to full term. The spontaneous bursting activity that characterizes cultures develops in parallel with the connectivity. The average number of inputs per neuron can be quantitatively determined in units of m0, the number of activated inputs needed to excite the neuron. For m0 = 10 we find that hippocampal neurons have on average 40-80 inputs while cortical neurons have 50-100, depending on neuronal density. The ratio of excitatory to inhibitory neurons is determined using the GABAA antagonist bicuculine. This ratio changes during development and reaches the final value at day 7-8, coinciding with the expected time of the GABA switch. For hippocampal cultures the inhibitory cells comprise about 30% of the neurons in the culture while for cortical cultures they are about 20%. Such detailed global information on the connectivity of networks in neuronal cultures is at present inaccessible by any electrophysiological or other technique.

Experimental setup

Novel approach: collective electric stimulation + gradual weakening of the network.



(a) Fluorescence image of a small region of the neural culture. The neural culture contains ~10^5 neurons, and we monitor the activity of a small region containing 500-1000 neurons. (b) Collective stimulation through bath electrodes. (c) Activity plot of the neural response. Black lines indicate those neurons that respond to the excitation. (d) Fluorescence signal for 3 neurons at increasing voltages. Vertical lines show the excitation time, and arrows the responding neurons.

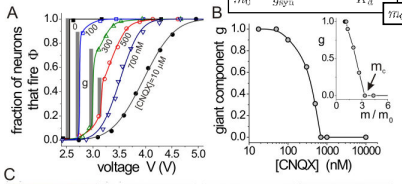


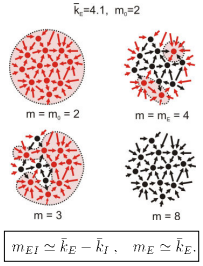
Examples of neural cultures. (a) Mature E19 hippocampal neurons. (b) E17 hippocampal neurons at DIV 3. (c) Mature E19 cortical neurons with homogeneous distribution. (d) Mature E19 cortical neurons with a clustered distribution. Scale bar is 100 μm.

Network response and giant component

(A) Response curves Φ(V) for a hippocampal network and for gradually higher concentration of CNQX. The grey bars show the size of the giant component g. (B) Corresponding size of the giant component as a function of [CNQX] (main plot) and as a function of the control parameter $m/m_0 = 1 + [CNQX]/K_D$ (inset). K_D=300 nM. **The control parameter quantifies the average connectivity of the network in units of m_0.** (C) Spatial coverage of the giant component (red) for the response curves shown in (A).

$$\frac{m}{m_0} = \frac{V_T}{g_{syn}} = 1 + \frac{[CNQX]}{K_d} \qquad m_0 \simeq 10$$



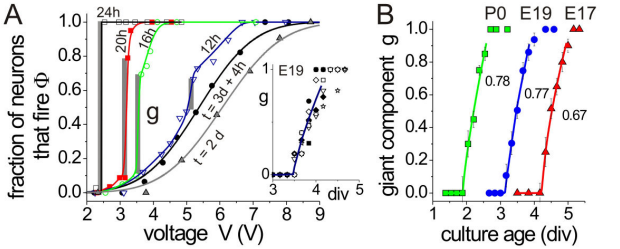


$$m_{EI} \simeq \bar{k}_E - \bar{k}_I \,, \quad m_E \simeq \bar{k}_E$$

Control parameter and m_0. Schematic disintegration of a network with only excitatory inputs, average connectivity k_E = 4.1 and m_0=2. All neurons that have at least m inputs fire and pass the signal (red), the rest remain inactive (black). A giant cluster (**giant component**) initially connects most of the network, and decreases in size as more neurons become inactive.

Development of connections

(A) Response curves Φ(V) for E19 hippocampal cultures at different developmental stages. The grey bars show the size of the giant component. Lines are a guide to the eye except for t=2 days and t=3 days + h that correspond to fits to error functions. Inset: size of the giant component as a function of time for 7 experiments with E19 cultures. The line is a power law fit of the averaged data. (B) Size of the giant component as a function of time for hippocampal cultures derived from brains at 3 different developmental stages: E17, E19, and P0. Lines are power law fits $g \sim |t - t_0|^{\gamma}$. The values indicate the critical exponent γ.



The timing of the emergence of the giant component is shifted according to the development of the embryonic brains. All cultures achieve full connectivity by E21-E22, about the expected time of birth.

During the development of the rat brain, a state of full connectivity is achieved close to delivery.

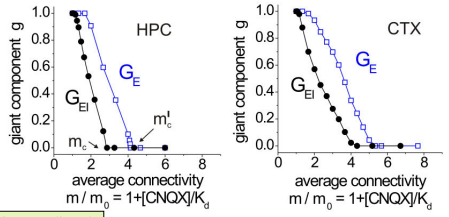
Average connectivity and amount of excitation

The value of m/m_0 at which the giant component disintegrates for G_E networks provides estimation of the average connectivity of the network. Cortical cultures have a higher connectivity than hippocampal ones.

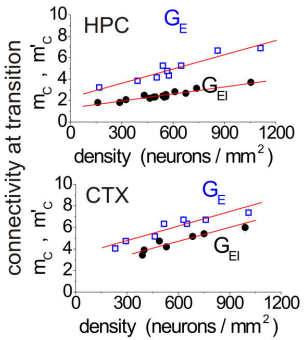
$$m_c = \frac{m_{KI}}{m_0} \,, \quad m'_c = \frac{m_K}{m_0} \,.$$

$$k_E \simeq m'_c m_0 \,, \quad \frac{\bar{k}_I}{\bar{k}_E} = 1 - \frac{m_c}{m'_c} \,.$$

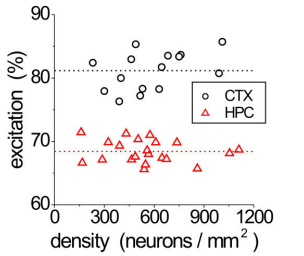
Average number of input connections	
HPC	CTX
40 - 80	50 - 100



The critical points m_c and m'_c increase linearly with the density of the neural culture.

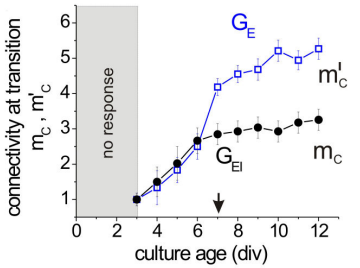


The critical points m_c and m'_c increase linearly with the density of the neural culture.

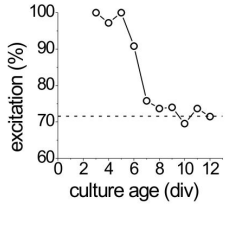


Emergence of inhibition

The evolution of the critical points m_C (G_{EI}) and m'_c (G_E networks) permits to identify the emergence of inhibition, which takes place at DIV 7 for E17 hippocampal cultures.



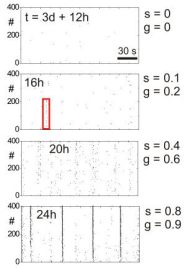
We can also quantify the increase of connectivity during development and the amount of excitation in the network.



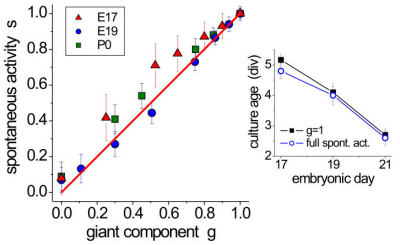
Spontaneous activity during development

The beginning of network bursting coincides with the formation of the giant component

Raster plots of spontaneous activity during the formation of the giant component for E19 cultures, measured at 4h interval starting at DIV 3.5. Each plot shows the activity of 400 individual neurons along 3 min. The red box outlines the largest fraction of neurons s that fired together.



Largest fraction of neurons in the network that show synchronized spontaneous activity as a function of the size of the giant component, and for E17, E19 and P0 cultures. The inset shows the time at which g=1 (squares) and time of emergence of spontaneous activity extending the entire network (circles) as a function of the embryonic day of the cultures.



Summary and conclusions

- **We have presented a novel experimental technique based on collective excitation of neurons. In combination with ideas of graph and percolation theory we are able to extract relevant information of the connectivity of the network, such as the average number of input connections, the amount of excitation, or the effect of neural density.**
- **We can also follow the development of connections as the network maturates. We observed that the timing of the emergence of the giant component is shifted according to the development of the embryonic brains, and that GABA switch takes places around DIV 7 for E17 cultures.**

References

(1) I. Breskin, J. Soriano, T. Tlusty, E. Moses, ***Percolation in Living Neural Networks***, Phys. Rev. Lett. **97**, 188102 (2006).
(2) J.-P. Eckmann, O. Feinermann, L. Gruendlinger, E. Moses, J. Soriano, T. Tlusty, ***The Physics of Living Neural Cultures***, Phys. Reports **449**, 54 (2007).
(3) J. Soriano, M. Rodriguez-Martinez, T. Tlusty, E. Moses. ***Development of Input Connections in Neural Cultures***, Proc. Natl. Acad. Sci. USA (in press).