Functional Connectivity Maps In Hippocampal Cultures Coupled To High Resolutions MEAs Underlie Structural Connectivity

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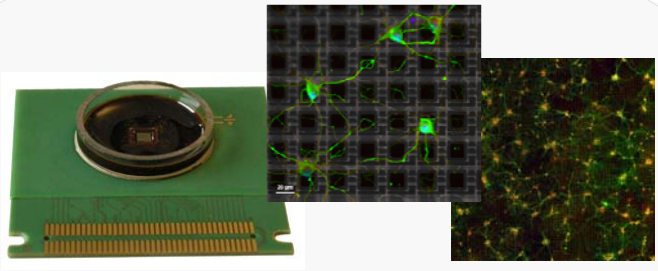
ABSTRACT

Identification of causal relationships between pairs of neurons plays an important role in the study of synaptic interactions at population level. We evaluated the functional connectivity by applying both a **cross-correlation** (CC) [1] and an **Entropy** based method [2] to low-density cultures of hippocampal neurons coupled to high resolution CMOS Micro Electrode Arrays [3] which allow a multi site acquisition at high-spatial as well as high-temporal resolution. We show that the connectivity estimated by CC compares qualitatively with the actual connectivity observed by means of fluorescent images.



DATA ANALYSIS

Spiking and bursting activity is identified by a threshold-based algorithm analyzing the extracellular signal recorded by each microelectrode: only the information regarding the position is stored. Then, statistical methods to estimate the functional connectivity are applied.

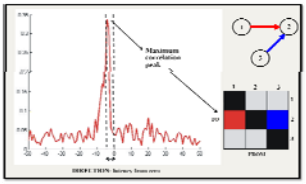
MATERIALS

Electrophysiological activity of populations of neurons extracted from hippocampus of rat embryos are recorded. Low-density cultures are plated over 64x64 CMOS-MEAs characterized by electrodes diameters of 20 µm and inter-electrode distances of 42 µm (pitch-to-pithch).

FUNCTIONAL CONNECTIVITY

The **FC** measures patterns of deviations from statistical independence and is fundamental to better understand the dynamics of a wide variety of complex neuronal networks.

CROSS-CORRELATION



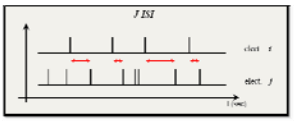
$$C(\tau)=\frac{1}{\sqrt{N_x N_y}}\sum_{s=1}^{N_x}\sum_{t_i=(\tau-\frac{\Delta\tau}{2})}^{\tau+\frac{\Delta\tau}{2}}x(t_s)y(t_s-t_i)$$

t_s = timing of a spike in the x train
N_x, N_y = total number of x and y train.

The **connection strength** is defined based on the maximum peak (C_{peak}) of the cross-correlogram function $C_{xy}(\tau)$. The **direction** is obtained thanks to the peak latency from the center of the cross-correlogram.

To take into account only statistically relevant C_{peak}, we set empirically the constraint on the minimum number of events (7) contributing to the C_{peak} and to the whole (10) Cross-Correlogram.

JOINT ENTROPY



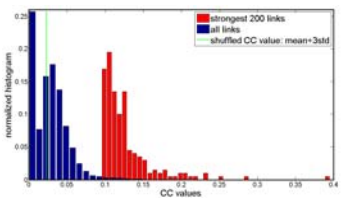
$$H(jISI)=-\sum p(jISI)\cdot\log_2[p(jISI)]$$

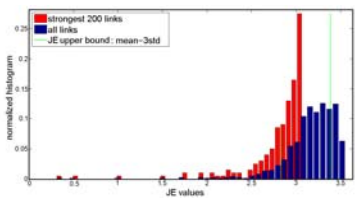
Entropy based on joint inter spike interval (J-ISI) is evaluated to assess causality among couples of channels [2].

We computed entropy for all the possible couple of channels considering the relative distribution of J-ISI, p(jISI). Strongest causal connections are associated to lower values.

THRESHOLDING PROCEDURE

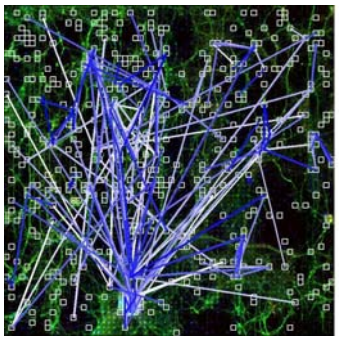
The value used to threshold the Connectivity Matrix is a crucial parameter. Heuristically, we chose absolute values to account for the strongest K-links among the possible N-links (K<N). The selected CC-thresholds were compared to a "**lower bound**" obtained evaluating the CC on shuffled spike trains (maintaining the original ISIs distribution). JE-thresholds were compared to an "**upper bound**" derived from the mathematical properties of the entropy.

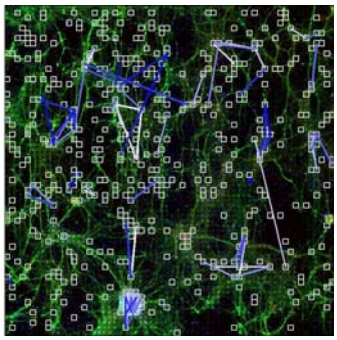




SPATIO-TEMPORAL FILTER

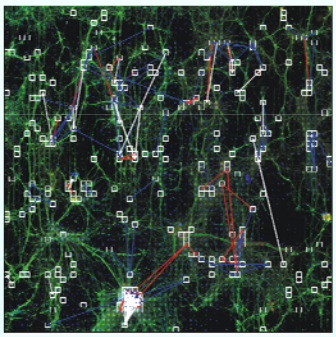
If the mean propagation velocity is not physiologically plausible, the link is discarded (panel B). We assumed a maximum propagation velocity (400 mm/s) [4]. Link strength are coded by the color intensity.

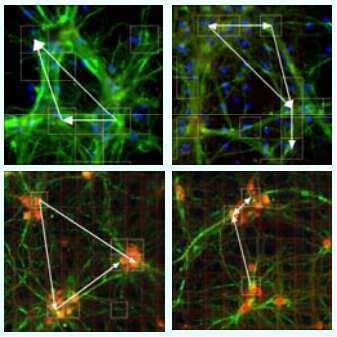




RESULTS

Links inferred by JE (blue), by CC (red) and the ones commonly identified (white) are compared with the morphology extracted by fluorescent technique. Results regarding two experiments are reported. Maps reveal a resolution scale not allowed by commercial MEAs: they allow to focus on microcircuit almost at a single cell precision.





CONCLUSIONS

Both methods (with the support of the filter) infer FC maps from which micro and macro circuits may be recognized that well match the underlining morphology. We speculate that CC is better suited to detect local neuronal interactions while JE is more indicated to recognize longer paths.

References

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[2] M. Garofalo, T. Nieus, P. Massobrio, S. Martinoia, "*Evaluation of the Performance of Information Theory-Based Methods and Cross-Correlation to Estimate the Functional Connectivity in Cortical Networks*", *PLoS ONE 4(8): e6482, August 2009.*
[3] Berdondini L, Imfeld K, Maccione A, Tedesco M, Neukon S, Koudelka-Hep M & Martinoia S. "*Active pixel sensor array for high spatio-temporal resolution electrophysiological recordings from single cell to large scale neuronal networks*". Lab on a Chip, 9, 2644-51 (2009)
[4] Bonifazi, P., M.E. Ruaro, and V. Torre, "*Statistical properties of information processing in neuronal networks. European Journal of Neuroscience*", 2005. 22(11): p. 2953-64.