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.

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

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

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

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.

RESULTS
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

MATERIALS
Electrophysiological activity of populations of neurons extracted from hippocampus of rat embryos are recorded. Low-density cultures are plated over 6x4 CMOS-MEAs characterized by electrodes diameters of 20 μm and inter-electrode distances of 42 μm (pitch-to-pitch).

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.