COMPUTATIONAL TOOL FOR COMPARING DEVELOPMENT OF CELLULAR-SCALE NETWORK ACTIVITY FROM MICROELECTRODE ARRAY (MEA) RECORDINGS OF 2D NEURONAL CULTURES AND 3D HUMAN CEREBRAL ORGANOIDS

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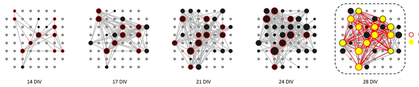
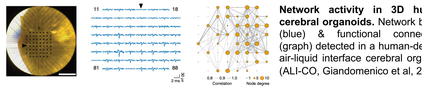
INTRODUCTION

Microelectrode Array (MEA) Recordings Reveal Cellular-Scale Network Activity



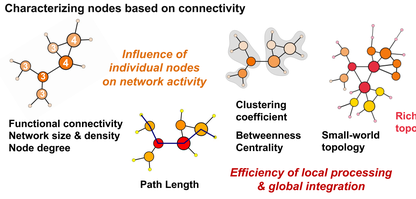
Modeling development of functional connectivity in vitro. Studies in mouse cortical (Downes et al, 2012) and hippocampal (above, Schroeter et al, 2015) cultures infer functional connectivity from correlated activity in MEA recordings and track network development including hubs.



Network activity in 3D human cerebral organoids. Network bursts (blue) & functional connectivity (graph) detected in a human-derived air-liquid interface cerebral organoid (ALI-CO, Giandomenico et al, 2019).

Network topology reveals patterns underlying efficiency of cellular-scale information processing

Characterizing nodes based on connectivity

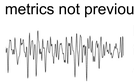


AIM

Create a tool to compare cellular-scale neuronal network activity and topology across age and genotype in 2D or 3D mouse or human neuronal cultures.

METHODS

We have created a MATLAB-based diagnostic tool for batch analysis of network-level effects in MEA recordings. The tool, MEA-NAP, includes graph theoretical metrics from the Brain Connectivity Toolbox (Rubinov & Sporns, 2010) and new network metrics not previously applied to cellular-scale neuronal activity.



Inputs to the pipeline: (1) raw voltage data from Multi Channel Systems single-well or Axion Biosystems multi-well MEA systems, converted to .mat files & (2) spreadsheet with filenames, age & group information.

Outputs from the pipeline include figures, statistics, and comparison plots organized in a convenient folder structure by pipeline step, group & individual recording.

Sample output folder for experiment comparing 2D iPSC-derived neuronal cultures in two groups (high & low density). Subfolders include plots for each recording & group comparison plots. Inset, sample network plots for an individual MEA recording.

For detailed methods, references and source code, visit:

https://sand-lab.github.io/

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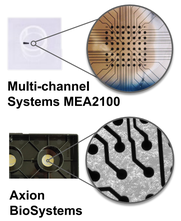
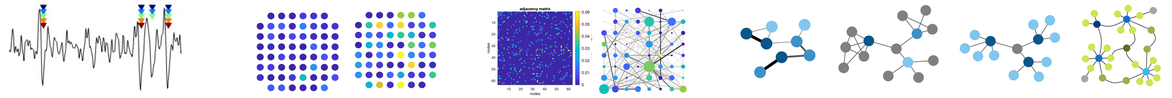
OVERVIEW OF MEA NETWORK ANALYSIS PIPELINE (MEA-NAP)

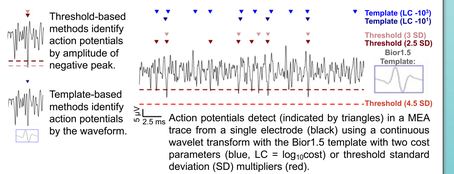


1. SPIKE DETECTION → **2. COMPARE NEURONAL ACTIVITY** → **3. INFER FUNCTIONAL CONNECTIVITY** → **4. COMPARE NETWORK ACTIVITY** → **5. FEATURE SELECTION**

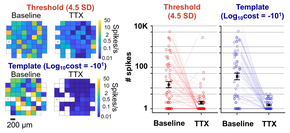


1. SPIKE DETECTION

Template-based methods for spike detection

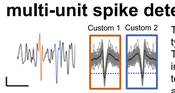


Validation of spike detection methods with TTX



Application of tetrodotoxin (TTX) blocks Na+ channels when neurons fire action potentials (AP) preventing further AP. Template-based method shows more AP in baseline and fewer AP in TTX—a higher sensitivity & specificity than the best performing threshold method.

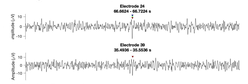
New electrode-specific custom templates & multi-unit spike detection method



The morphology of the AP waveform depends on the type of neuron and orientation relative to the electrode. To improve spike detection, our method first identifies AP in each electrode and creates one or more custom templates for each electrode. This template is then used along with the built-in MATLAB templates (e.g., Biot1.5, Biot1.3) to identify AP in each electrode.

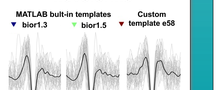
1. SPIKE DETECTION VALIDATION TOOLS

Evaluating spike detection with single action potential resolution

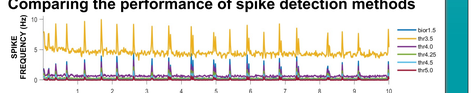


MEA-NAP produces sample voltage traces with spikes detected by different methods (colored triangles) in electrodes (left) and average spike waveforms by method (black) for spikes detected (gray) aligned by the negative peak (right).

Comparing spike waveforms



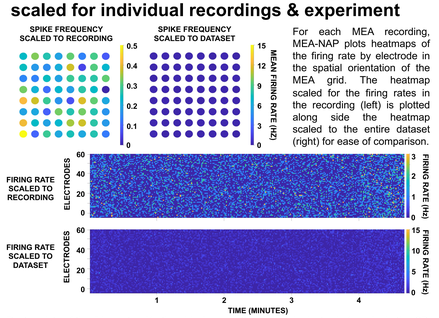
Comparing the performance of spike detection methods



MEA-NAP plots the running average of the spike frequency for each method (colored traces, see legend for method). In this 10-second-long sample of a MEA recording from a 3D human cerebral organoid, both template- (biort1.5 template) and threshold-based (3.5-5 SD) methods reveal periodic increases in action potentials firing. The template-based method (biort1.5, dark blue) is highly sensitive and specific for spikes compared to the thresholds applied.

2. NEURONAL ACTIVITY IN INDIVIDUAL NETWORKS

Spatial & temporal resolution for firing rates scaled for individual recordings & experiment

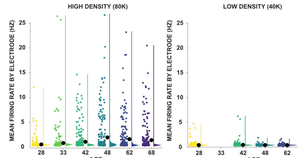


For each MEA recording, MEA-NAP plots heatmaps of the firing rate by electrode in the spatial orientation of the MEA grid. The heatmap scaled for the firing rates in the recording (left) is plotted along side the heatmap scaled to the entire dataset (right) for ease of comparison.

Raster plots of the firing rates (1s time bins) are plotted for each electrode (rows). In this 2D iPSC-derived cortical culture, the raster plot scaled for the recording (top) reveals variation in firing rate (0-3 Hz) within and between electrodes. The raster plot scaled for entire dataset (below) shows that the firing rate is low relative to other MEA recordings in this experiment.

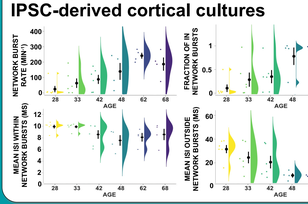
2. COMPARE NEURONAL ACTIVITY

Development of neuronal activity in 2D human IPSC-derived cortical cultures



MEA-NAP reveals effect of cell density on the increase in neuronal firing in human IPSC-derived cultures from days-in-vitro (DIV) 28-68 plated with either 80K (left) or 40K (right) neurons. Half violin plots show mean firing rate in individual electrodes with mean+sem (black) and density curves showing the distribution.

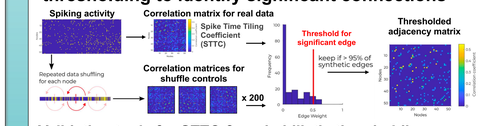
Development of network bursts in 2D human IPSC-derived cortical cultures



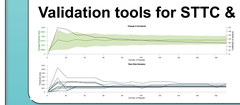
MEA-NAP plots reveal an age-related increase in network bursts over DIV 28-68. The mean inter-spike interval (ISI) within and outside of network bursts decreases as the network activity becomes more synchronized.

3. INFER FUNCTIONAL CONNECTIVITY

Spike time tiling coefficient (STTC) & probabilistic thresholding to identify significant connections



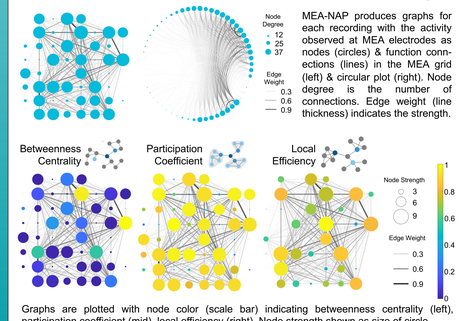
Validation tools for STTC & probabilistic thresholding



Functional connectivity (above) is inferred from pairwise comparisons of spiking activity between electrodes with the STTC (Cutts & Eglen, 2014). MEA-NAP produces plots for each recording (left) to assess the effect of the number of iterations on edge threshold (shown for 200 shuffle controls).

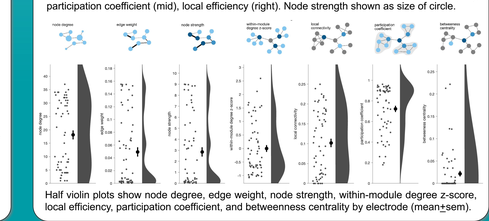
4. NETWORK TOPOLOGY IN INDIVIDUAL NETWORKS

Network features plotted for each MEA recording



MEA-NAP produces graphs for each recording with the activity observed at MEA electrodes as nodes (circles) & function connections (lines) in the MEA grid (left) & circular plot (right). Node degree is the number of connections. Edge weight (line thickness) indicates the strength.

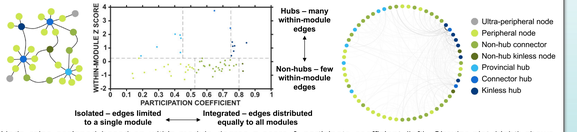
Graphs are plotted with node color (scale bar) indicating betweenness centrality (left), participation coefficient (mid), local efficiency (right). Node strength shown as size of circle.



Half violin plots show node degree, edge weight, node strength, within-module degree z-score, local efficiency, participation coefficient, and betweenness centrality by electrode (mean±sem).

4. NEW NETWORK METRICS FOR CELLULAR-SCALE NETWORKS

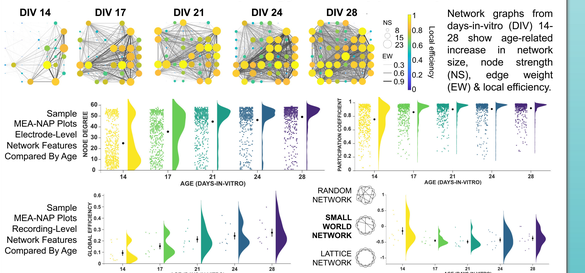
Node cartography reveals role of individual nodes in the network



Node roles assigned based on within-module degree z score & participate coefficient (left). Circular plot (right) shows connections between nodes. Adapted from methods for complex metabolic networks by Guimerà & Nunes Amara (2005) and fMRI brain networks by Shine et al. (2015; 2016).

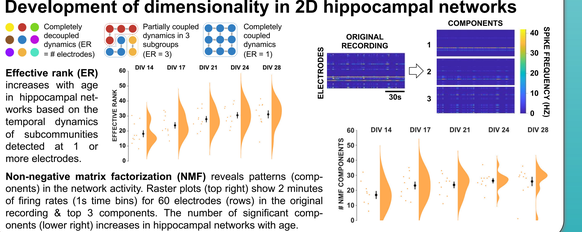
4. COMPARE NETWORK ACTIVITY BY AGE & GENOTYPE

Development of network topology in 2D hippocampal cultures



Network graphs from days-in-vitro (DIV) 14-28 show age-related increase in network size, node strength (NS), edge weight (EW) & local efficiency.

Development of dimensionality in 2D hippocampal networks

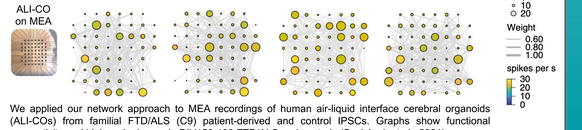


Effective rank (ER) increases with age in hippocampal networks based on the temporal dynamics of subcommunities detected at 1 or more electrodes.

Non-negative matrix factorization (NMF) reveals patterns (components) in the network activity. Raster plots (top right) show 2 minutes of firing rates (1s time bins) for 60 electrodes (rows) in the original recording & top 3 components. The number of significant components (lower right) increases in hippocampal networks with age.

CLINICAL APPLICATIONS

Network activity in 3D human cerebral organoid disease models



We applied our network approach to MEA recordings of human air-liquid interface cerebral organoids (ALI-COs) from familial FTD/ALS (C9) patient-derived and control IPSCs. Graphs show functional connectivity and high node degree in DIV150-193 FTD/ALS and controls (Szebényi, et al., 2021).

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