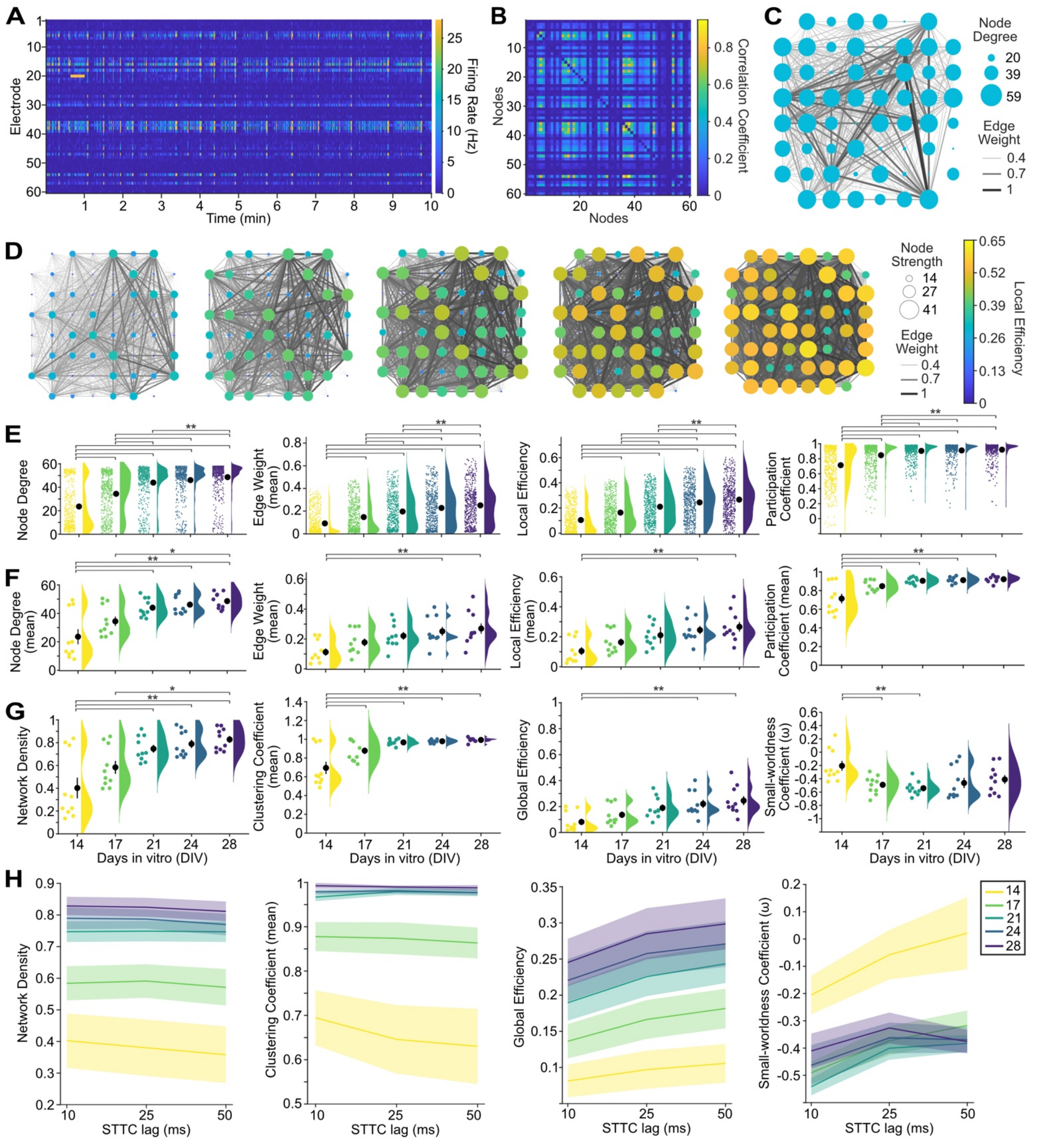
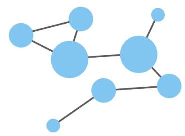
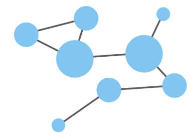
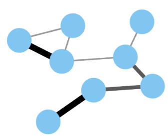
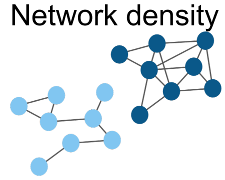
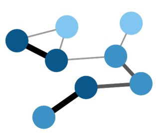
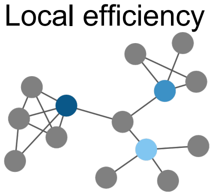
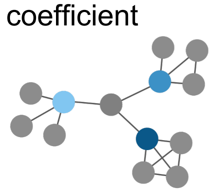
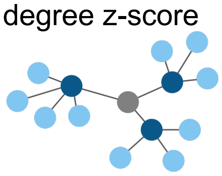
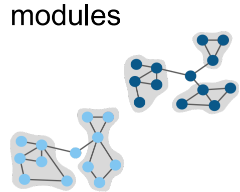
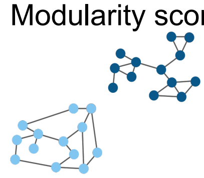
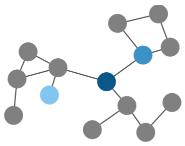
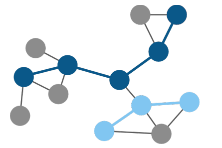
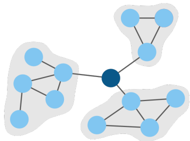
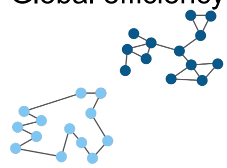
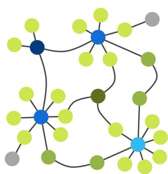
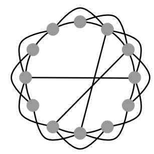
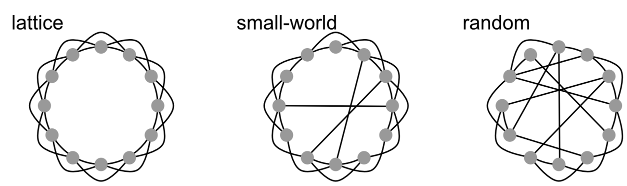
Supplementary material



Supplemental Figure S1. Development of functional connectivity and network topology in 2D murine hippocampal cultures. A. Representative raster plot of spontaneous activity in a 10-minute microelectrode array (MEA) recording from primary mouse hippocampal culture. **B.** Adjacency matrix shows correlation coefficient (spike time tiling coefficient, STTC) for significant edges after probabilistic thresholding for recording in A. **C.** Graph of functional connectivity for recording in A. Nodes (circles) represent the activity observed at individual electrodes in the spatial arrangement of the MEA. Number of connections shown as node degree (circle size) and strength of connectivity as edge weight (line thickness). **D.** Development of functional connectivity in representative hippocampal cultures from days-in-vitro (DIV) 14-28 including increase in node strength (circle size), edge weight (line thickness), and local efficiency (circle color). **E.** Comparison of nodal-level network metrics for electrodes (colored circles) from hippocampal cultures (n=10) for node degree, mean edge weight (per node), local efficiency, and participation coefficient. Scatter plots with mean (black circles) ± SEM (error bars) and density curve for DIV 14-28. **F.** Comparison of recording-level network metrics (colored circles) for mean node degree, mean edge weight, mean local efficiency, and mean participation coefficient from DIV 14-28. **G.** Comparison of recording-level network metrics including network density, mean clustering coefficient, global efficiency, and small-worldness from DIV 14-28. **H.** Comparison of recording-level network metrics by STTC lag and developmental age (color, DIV 14-28). Means (lines) ± SEM (shading). For panels E-G, a one-way ANOVA ($p<0.01$ for all plots) followed by the Tukey-Kramer method to calculate p-values adjusted for multiple post-hoc pairwise comparisons (** $p \leq 0.01$, * $p<0.05$).

Supplemental Table 1. Functional connectivity, network topology, and network dynamic metrics in MEA-NAP

Nodal-level Feature	Description	Recording-level Feature	Description
Basic features of the network			
Node degree 	Number of connections (edges) with other nodes in the network. Highly connected nodes may have more influence on network activity.	Network size 	Number of active electrodes (defined by a minimum number or frequency of spikes detected).
Edge weight 	Strength of connectivity between two nodes. Calculated using the spike-time tiling coefficient (*Cutts & Eglen, 2014*)	Network density 	Number of edges as a proportion (%) of the total possible edges that can be formed in the network.
Node strength 	Sum of the edge weights for each node.		
Local processing in the network			
Local efficiency 	Efficiency defined at the level of individual nodes. The inverse of path length of the subgraph generated by removing the index node and its edges. *(Latora & Marchiori, 2001)*	Clustering coefficient 	Probability that two nodes each directly connected to a third node will also be directly linked to each other.
Within-module degree z-score 	Measure of how well-connected a node is to other nodes in the same module. Higher values indicate higher intramodular node degree. *(Guimerà & Nunes Amaral, 2005)*	Number of modules 	Number of subsets of nodes into which the network can be decomposed, where nodes in each subset are more densely connected to each other than to nodes in other subsets. *(Brandes et al., 2008)*
Affiliation vector	Vector containing the number of the module to which each node belongs.	Modularity score 	A value between -0.5 and 1 that describes how well a network has been partitioned. *(Lancichinetti & Fortunato, 2012)*
Global processing and nodal roles in the network			
Betweenness centrality	Number of times a node lies on the shortest path between	Path length	Characteristic path length is the minimum number of edges

	any two nodes in a network. *(Brandes, 2001)*		required to link any two nodes in the network averaged across nodes.
Participation coefficient 	Measure of how well-distributed a node's edges are among different modules. *(Guimerà & Nunes Amaral, 2005*)	Global efficiency 	Efficiency of parallel information transfer between nodes in the network. Inverse of characteristic path length. *(Latora & Marchiori, 2001)*
Node cartography group proportions 	Each node is assigned a role by node cartography group. *(Guimerà & Nunes Amaral, 2005)* 1. Peripheral nodes 2. Non-hub connectors 3. Non-hub kinless nodes 4. Provincial hubs 5. Connector hubs 6. Kinless hubs	Small-world coefficient, *Method 1* (σ) 	Network topology with clusters of nodes connected to other clusters via hub nodes. This reduces path length and facilitates both local and global information processing. Calculated as clustering coefficient divided by characteristic path length. *(Humphries et al, 2006; Humphries & Gurney, 2008)*
Hub score	Hubs are nodes with high centrality in the network. Nodes are ranked based on node strength, betweenness centrality, local efficiency, and participation coefficient. Hubs rank in the top 10% of nodes in 3 or 4 of these features. (*Schroeter et al., 2015*)	Small-world coefficient, *Method 2* (ω) 	Calculated using the normalized clustering coefficient and path length. Small-world network structure is at the midpoint (0) between a lattice (-1) and random (1) network structure. *(Telesford et al., 2011)*
Network dynamics			
Non-negative matrix factorization (NMF)	Dimensionality reduction approach identifies patterns of activity in the network and the number of electrodes participating in each pattern.	Effective Rank	Dimensionality reduction approach calculates the number of sub-communities within the network. *(Roy & Vetterli, 2007)*

Supplemental Table 2. Code from other sources incorporated in MEA-NAP			
Reference(s)	Description	Location in MEA-NAP	Source code
Methods - Spike detection			
Nenadic Z & Burdick JW (2005). Spike detection using the continuous wavelet transform. *IEEE T Bio-med Eng*, 52, 74-87. Benitez R & Nenadic Z (2008). Robust unsupervised detection of action potentials with probabilistic models. *IEEE T Bio-med Eng*, 55(4), 1344-1354.	Continuous wavelet transform (CWT) method for template-based spike detection using the MATLAB function detect_Spikes_wavelet.m	detectSpike.m, getTemplate.m, customWavelet.m, detectSpikesWavelet.m (*optional step in MEA-NAP*)	http://cbmspc.eng.uci.edu/SOFTWARE/SPIKEDETECTION/detect_spikes_wavelet.m.
Lieb F et al. (2017). A stationary wavelet transform and a time-frequency based spike detection algorithm for extracellular recorded data. *J Neural Eng*, 14(3), 036013.	Stationary wavelet transform (SWTTEO) method for template-based spike detection.	detectSpike.m (*optional step in MEA-NAP*)	https://github.com/flieb/SpikeDetection-Toolbox
Methods - Burst analysis			
Bakkum DJ, et al. (2014). Parameters for burst detection. *Front Comput Neurosci*, 7(193).	Method for burst detection. Based on ISI_N burst detector (Bakkum, 2013) using BurstDetectISIn.m & HistogramISIn.m (modified)	BurstDetectISIn.m, getISInTh.m	https://www.frontiersin.org/articles/file/downloadfile/61635_supplementary-materials_presentations_1_pdf/octet-stream/Presentation%201.PDF/1/61635
Methods - Functional connectivity			
Cutts CS & Eglen SJ (2014). Detecting pairwise correlations in spike trains: An objective comparison of methods and application to the study of retinal waves. *J Neurosci*, 34(43), 14288–14303.	Spike-time tiling coefficient (STTC)	get_sttc.m	https://github.com/CCutts/Detecting_pairwise_correlations_in_spike_trains/blob/master/spike_time_tiling_coefficient.c
Methods - Network features			
Rubinov M & Sporns O (2010). Complex network measures of brain connectivity: Uses and interpretations. *NeuroImage*, 52(3), 1059–1069.	Brain Connectivity Toolbox (BCT) for calculating graph theoretical metrics and null models.	Functions in 2019_03_03_BCT folder, CC_PL_SW folder	http://www.brain-connectivity-toolbox.net/

Pedersen M et al. (2019). Reducing module size bias of participation coefficient. BioRxiv. doi: 10.1101/747162. Retrieved December 8, 2021.	Normalizing the participation coefficient using random networks to preserve degree distribution	participation_coef_norm.m	https://github.com/omidvarnia/Dynamic_brain_connectivity_analysis
Bettinardi RG (2017). getCommunicability(W,g,nQexp) MATLAB Central File Exchange. Retrieved June 6, 2022.	Communicability function. (Used in fcn_find_hubs_wu.m for ExtractNetMet.m)	getCommunicability.m	https://www.mathworks.com/matlabcentral/fileexchange/62987-getcommunicability-w-g-nqexp
Methods - Statistics			
Trujillo-Ortiz A., et al. (2004). RMAOV1:One-way repeated measures ANOVA. MATLAB Central File Exchange. Retrieved August 3, 2023.	One-way repeated measures ANOVA	RMAOV1.m	https://www.mathworks.com/matlabcentral/fileexchange/5576-rmaov1
Schurger A (2005). Two-way repeated measures ANOVA. MATLAB Central File Exchange. Retrieved August 3, 2023.	Two-factor, within-subject repeated measures ANOVA	rm_anova2.m	https://www.mathworks.com/matlabcentral/fileexchange/6874-two-way-repeated-measures-anova
Tools - GUI			
Hoelzer S (2010). Progress bar. MATLAB Central File Exchange. Retrieved December 8, 2021.	Progress bar	progressbar.m	https://www.mathworks.com/matlabcentral/fileexchange/6922-progressbar
Tools - Plotting			
Marsh G (2016). LOESS regression smoothing. MATLAB Central File Exchange. Retrieved June 23, 2023.	Smoothing function using LOESS (locally weighted regression fitting using a 2nd order polynomial)	fLOESS.m, getISInTh.m	https://www.mathworks.com/matlabcentral/fileexchange/55407-loess-regression-smoothing
Lee T (2006). Kernel density estimation of 2 dim with SJ bandwidth. MATLAB Central File Exchange. Retrieved June 23, 2023.	Kernel density estimator with Sheater Jones (SJ) bandwidth	bandwidth_SJ.m, KDE2.m	https://www.mathworks.com/matlabcentral/fileexchange/10921-kernel-density-estimation-of-2-dim-with-sj-bandwidth

Botev Z (2015). Kernel density estimator. MATLAB Central File Exchange. Retrieved June 23, 2023.	Faster kernel density estimator	improvedSJkde.m	https://www.mathworks.com/matlabcentral/fileexchange/14034-kernel-density-estimator
Thyng KM, et al. (2016). True colors of oceanography. *Oceanography*, 29(3), 10.	Colormap generator	cmocean.m	https://matplotlib.org/cmocean/
Kumpulainen K (2016). tight_subplot. MATLAB Central File Exchange. Retrieved June 19, 2023.	Creates axes subplots with adjustable margins and gaps between the axes	tight_subplot.m	https://www.mathworks.com/matlabcentral/fileexchange/27991-tight_subplot-nh-nw-gap-marg_h-marg_w
Schwizer J (2015). Scalable vector graphics export of figures (fig2svg). GitHub. Retrieved June 16, 2022.	Converts MATLAB plots to the scalable vector format (SVG)	Functions in fig2svg folder	https://github.com/jschwizer99/plot2svg
Campbell R (2020). notBoxPlot. GitHub. Retrieved December 8, 2021.	Plots raw data as a jitter, mean, s.e.m., and 95% confidence intervals (modified)	notBoxPlotRF.m	https://github.com/raacampbell/notBoxPlot

Supplemental Table 3. Comparison with other publicly available MEA analysis or functional connectivity tools

	Adapted for MEA data	Spike Detection	Neuronal activity comparison	Inferring functional connectivity	Network metrics	Statistical analysis	Visualization & GUI
MEA-NAP	●	●	○	●	●	●	○
Brain Connectivity Toolbox				●	●	●	
MEA-ToolBox	●	○	○	○			○
MEAnalyzer	●	○	○	○	○		○
meaRtools	●		○	○		●	
BSMART				○	●		
ToolConnect	●			○	●		○
SPICODYN	●		○	○	○		○

Table Legend: Closed circle = many features; Open circle = limited features

Supplemental Table 4. Publicly available MEA analysis or functional connectivity toolboxes	
Method	Features
MEA data analysis tools	
MEA-ToolBox (MATLAB)	Source: https://github.com/DrJPFrimat/MEA-ToolBox Features: ● File conversion & filtering of raw MCS MEA data ● Threshold-based spike detection with artifact removal ● Single channel burst detection with max interval & log ISI method (from Cotterill et al., 2016) ● Network burst detection ● Cross-correlation to infer functional connectivity ● Synchronicity (pairwise) using ISI distance method ● Spike sorting ● GUI with data visualizations Reference: Hu M, Frega M, Tolner EA, van den Maagdenberg AMJM, Frimat JP, le Feber J. MEA-ToolBox: an Open Source Toolbox for Standardized Analysis of Multi-Electrode Array Data. Neuroinformatics. 2022 Oct;20(4):1077-1092.
MEAnalyzer (MATLAB)	Source: https://github.com/RDastgh1/MEAnalyzer Features: ● Spike detection with threshold method ● Spike and burst features ● Cross-correlation or overlapping spikes or bursts to infer functional connectivity ● Graph metrics (node degree, global efficiency, network size and network density) Reference: Dastgheyb RM, Yoo SW, Haughey NJ. (2020) MEAnalyzer - a Spike Train Analysis Tool for Multi Electrode Arrays. Neuroinformatics, 18(1):163-179.
meaRtools (R)	Source: https://cran.r-project.org/src/contrib/Archive/meaRtools/ Features: ● Spike features (no spike detection) ● Single channel burst features ● Burst and network burst features ● Spike-time tiling coefficient (mean per network) ● Entropy (mean per network) ● Mutual Information (pairwise comparison of patterns in spike trains) Reference: Gelfman S, Wang Q, Lu YF, Hall D, Bostick CD, Dhindsa R, Halvorsen M, McSweeney KM, Cotterill E, Edinburgh T, Beaumont MA, Frankel WN, Petrovski S, Allen AS, Boland MJ, Goldstein DB, Eglen SJ (2018). meaRtools: An R package for the analysis of neuronal networks recorded on microelectrode arrays. PLoS Comput Biol,14(10):e1006506.
FIND (previously MEA-	Source:

tools) (MATLAB)	https://web.archive.org/web/20060910130103/http://www.brainworks.uni-freiburg.de/projects/mea/meatools/install_instructions.html Features: ● Identification of local field potentials & extracellular spike times & waveforms (method not specified) ● Basic spike sorting with principal component analysis ● GUI with limited data visualizations Reference: Egert U, Knott T, Schwarz C, Nawrot M, Brandt A, Rotter S, Diesmann M. (2002) MEA-Tools: an open source toolbox for the analysis of multi-electrode data with MATLAB. J Neurosci Methods, 117(1):33-42. Meier R, Egert U, Aertsen A, Nawrot MP. (2008) FIND--a unified framework for neural data analysis. Neural Netw, 21(8):1085-93.
MEA Viewer (Python)	Source: https://github.com/dbridges/mea-tools Features: ● Spike detection with threshold method ● GUI to view and examine spike detection Reference: Bridges DC, Tovar KR, Wu B, Hansma PK, Kosik KS. (2018). MEA Viewer: A high-performance interactive application for visualizing electrophysiological data. PLoS One, 13(2):e0192477.
McsMatlabDataTools (MATLAB)	Source: https://github.com/multichannelsystems/McsMatlabDataTools Features: ● Imports data from Multi-Channel System ● Visualization tools for data Reference: Armin Walter (2022). McsMatlabDataTools, GitHub.
Multiwell Analyzer (Windows application)	Source: https://www.multichannelsystems.com/software/multiwell-analyzer Features: ● For MCS multi-well MEA data ● Spike detection with threshold or slope method ● Single-channel and network burst detection Reference: Multi-channel Systems software (publicly available)
SPICODYN (C/Visual Studio)	Source: https://www.nitrc.org/projects/spicodyn/ Features: ● Spike detection with threshold methods ● Burst detection ● Infer functional connectivity with transfer entropy method ● Graph theoretical metrics (degree, path length, clustering coefficient, hubs, small-world index) ● Visualization tools in GUI Reference: Pastore VP, Godjoski A, Martinoia S, Massobrio P. (2018) SPICODYN: A Toolbox for the Analysis of Neuronal Network Dynamics and Connectivity from Multi-Site Spike Signal Recordings. Neuroinformatics, 16(1):15-30.

SPKtool (MATLAB)	Source: https://spktool.sourceforge.net/ Features: ● Spike detection via threshold method ● Spike features ● Spike sorting ● Cross-correlograms Reference: Liu X, Wu X, Liu C (2011). SPKtool: An open source toolbox for electrophysiological data processing," 2011 4th International Conference on Biomedical Engineering and Informatics (BMEI), Shanghai, China, 2011, pp. 854-857.
SPYCODE (MATLAB)	Source: Bologna et al. (2010) requires prospective uses to email senior author to obtain code. Features: ● Spike detection with threshold methods and spike features ● Network spike and burst features ● Infer functional connectivity with cross-correlation and/or information theoretical approaches ● Neuronal avalanche detection (features within bursts) Reference: Bologna LL, Pasquale V, Garofalo M, Gandolfo M, Baljon PL, Maccione A, Martinoia S, Chiappalone M. (2010) Investigating neuronal activity by SPYCODE multi-channel data analyzer. Neural Netw, 23(6):685-97.
ToolConnect (C/Visual Studio)	Source: https://www.nitrc.org/projects/toolconnect/ Features: ● Infer functional connection from cross-correlation or partial-correlation methods ● Information theory (joint entropy, transfer entropy) based core algorithms ● Visualization tools in GUI Reference: Pastore VP, Poli D, Godjoski A, Martinoia S, Massobrio P. (2016) ToolConnect: a functional connectivity toolbox for in vitro networks. Front Neuroinform, 10:13.
Functional connectivity, network topology and network dynamics tools (not designed or specific to MCS 60-electrode or Axion 64-electrode MEA data analysis)	
Brain connectivity Toolbox (MATLAB)	Source: http://www.brain-connectivity-toolbox.net/ Features: ● Extensive graph theoretical metrics functions ● Tool commonly used for macroscale networks (especially neuroimaging) ● Statistical methods available through associated toolboxes (e.g., Zalesky et al., 2010. Network-based statistic: identifying differences in brain networks. Neuroimage, 53(4):1197-207) ● Requires knowledge of network neuroscience to use Reference: Rubinov M & Sporns O (2010). Complex network measures of brain connectivity: Uses and interpretations. NeuroImage, 52(3), 1059–1069.

BSMART (MATLAB/C)	Source: https://github.com/brain-smart/brain-smart.github.io Features: ● For 15 electrode, EEG, MEG or fMRI data ● Multivariate autoregressive (MAR) analysis ● Spectral analysis ● Granger causality ● Requires knowledge of network neuroscience to use Reference: Cui J, Xu L, Bressler SL, Ding M, Liang H. (2008) BSMART: a Matlab/C toolbox for analysis of multichannel neural time series. Neural Netw, 21(8):1094-104.
Chronux (MATLAB)	Source: http://chronux.org/ Features: ● Spike sorting ● Spectral analysis ● Coherence Reference: Bokil H, Andrews P, Kulkarni JE, Mehta S, Mitra PP. (2010). Chronux: a platform for analyzing neural signals. J Neurosci Methods, 192(1):146-51.
Elephant (Python)	Source: https://elephant.readthedocs.io/en/latest/modules.html Features: ● Designed for LFP and spike train analysis ● Spike train statistics ● Spike train correlation, synchrony, dissimilarity ● Require knowledge of python and network neuroscience to use with MEA data Reference: Denker M, Yegenoglu A, Grün S (2018). Collaborative HPC-enabled workflows on the HBP Collaboratory using the Elephant framework. Neuroinformatics, P19.
Graphene-Electrode-Seizures (MATLAB)	Source: https://github.com/BassettLab/Graphene-Electrode-Seizures Features: ● Designed for 16-electrode graphene MEA ● Non-negative matrix factorization to show seizure progression ● Limited documentation ● Code source from research article, requires knowledge of MATLAB to apply to MEA data Reference: Driscoll N, Rosch RE, Murphy BB, Ashourvan A, Vishnubhotla R, Dickens OO, Johnson ATC, Davis KA, Litt B, Bassett DS, Takano H, Vitale F. (2021) Multimodal in vivo recording using transparent graphene microelectrodes illuminates spatiotemporal seizure dynamics at the microscale. Commun Biol. 2021 Jan 29;4(1):136.
nSTAT (MATLAB)	Source: https://github.com/iahncajigas/nSTAT Features: ● Point process – generalized linear model for spike trains ● Requires knowledge of MATLAB to apply to MEA data

	Reference: Cajigas I, Malik WQ, Brown EN. (2012) nSTAT: open-source neural spike train analysis toolbox for Matlab. J Neurosci Methods, 211(2):245-64.
STAToolkit (MATLAB)	Source: http://neuroanalysis.org/ (*unable to access code from link*) Features: ● Information-theoretic methods ● Entropy-based spike train analysis methods ● Requires knowledge of ? to apply to MEA data Reference: Goldberg DH, Victor JD, Gardner EP, Gardner D. (2009) Spike train analysis toolkit: enabling wider application of information-theoretic techniques to neurophysiology. Neuroinformatics, 7(3):165-78.

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