

SUPPLEMENTARY MATERIALS

Flexibility of *in vitro* cortical circuits influences resilience from microtrauma

Modupe A. Adegoke¹, Olivia Teter¹, David F. Meaney^{1,2*}

¹Department of Bioengineering, School of Engineering and Applied Sciences, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

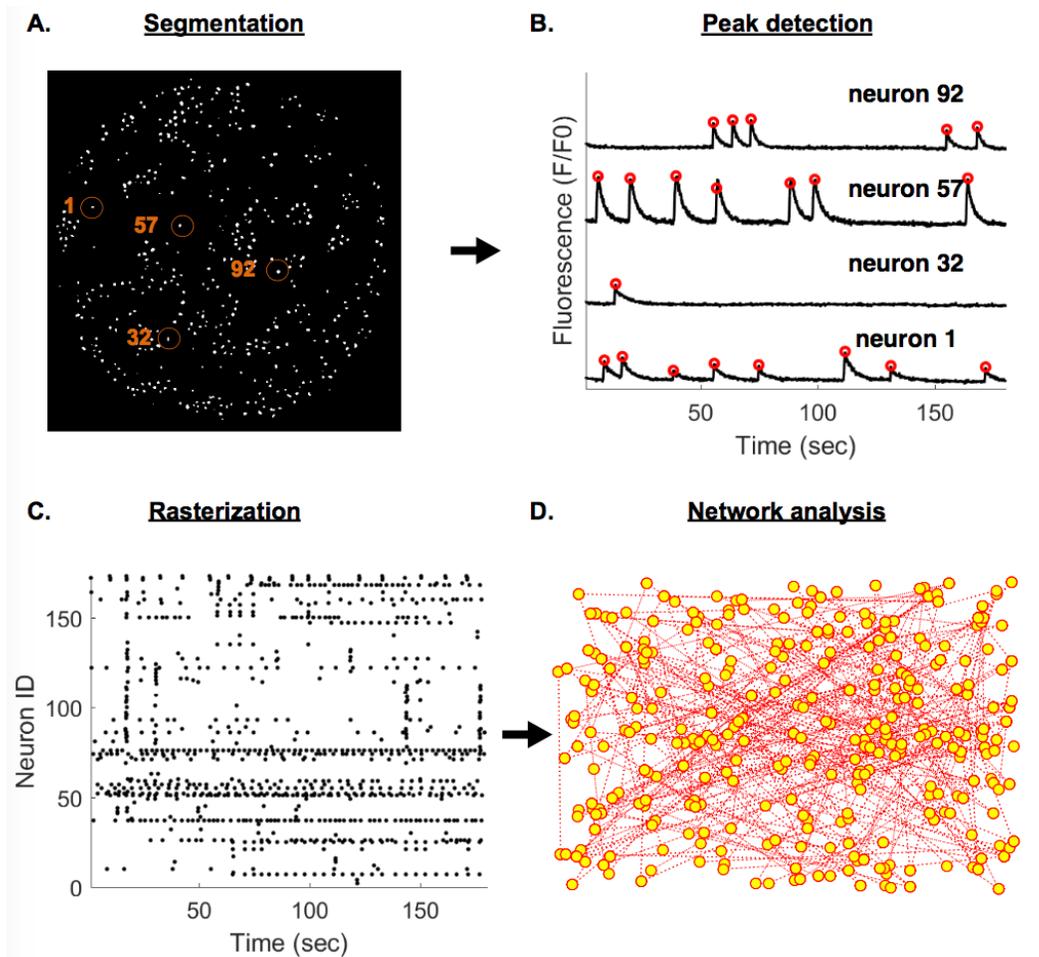
²Department of Neurosurgery, Penn Center for Brain Injury and Repair, Perelman School of Medicine, University of Pennsylvania, Philadelphia, Pennsylvania, United States of America

*** Correspondence:**

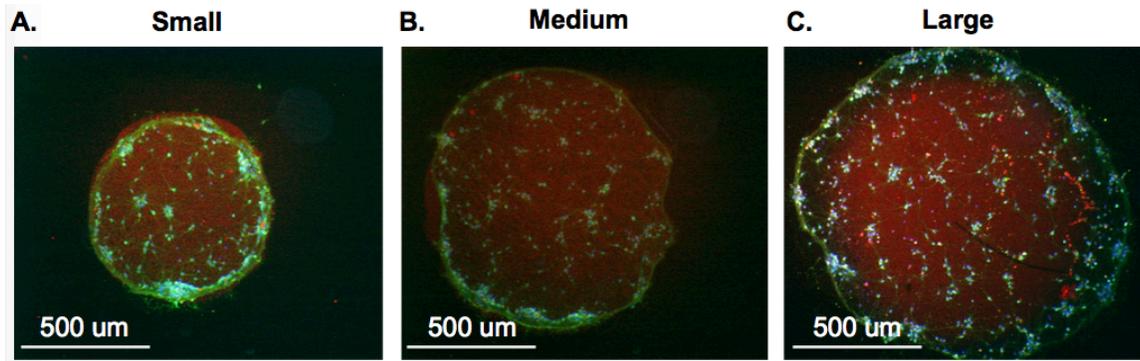
Corresponding Author

dmeaney@seas.upenn.edu

Keywords: traumatic brain injury, neuronal networks, susceptibility, calcium imaging, connectivity, circuit scaling. (Min.5-Max. 8)

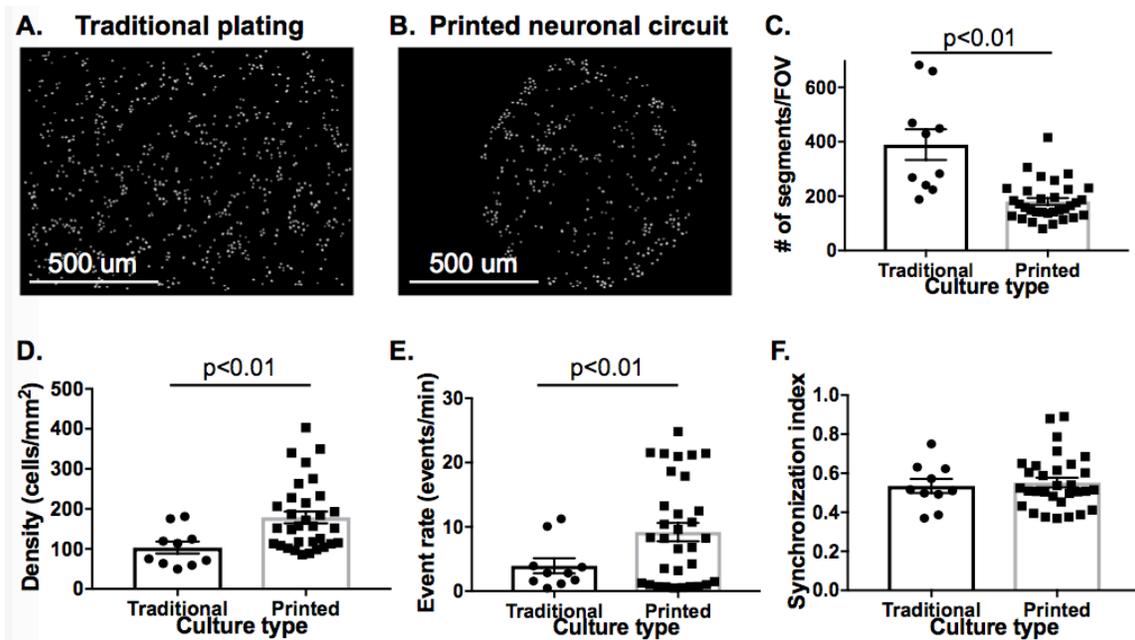


Supplementary Figure 1: Diagram of analysis pipeline. **A.** Manual segmentation of the maximum projection file for each .tiff stack of Ca^{2+} activity fluorescence yielded a binary mask where single neurons are represented as individual regions of interest (ROIs, orange circles). **B.** Single-cell Ca^{2+} transients were detected accurately using a MATLAB-based analysis system comparing brief segments of the fluorescence trace with a library of transient traces. **C.** Raster plots summarizing activity data from all cells within one island allow for quick visualization of activity patterns; each dot marks a detected transient. **D.** Functional connectivity networks constructed based on pairwise phase synchronization provide further information about the functional state of island circuits (yellow circles – nodes; red lines – binary pairwise functional connection).

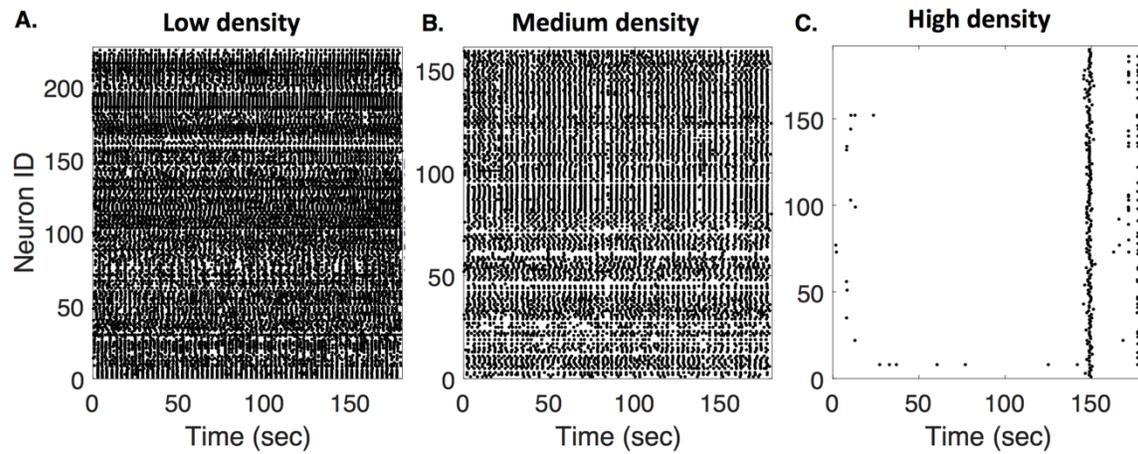


— Laminin — Map2 — Hoechst

Supplementary Figure 2: Immunolabeling of cell distribution in neuronal islands. Neurons selectively attached and migrated onto restricted areas of Laminin and PDL coating (red, Rb~Laminin) in patterned islands of different diameters (small: 750um; medium: 1,000um; large: 1,300 um). By 14DIV, cell bodies were distributed evenly across the island surface as evidenced by the DNA-binding stain Hoechst (blue), and connected through a well-developed network of neuronal projections (green, Ms~MAP2).



Supplementary Figure 3: Comparison between traditionally-plated dissociated cultures and neuronal islands. A-B. Segmentation masks corresponding to traditionally-plated dissociated culture and an island culture indicate overall even distribution of cells on both platforms by 10 DIV. C-F. Summary statistics from 10 traditional cultures and 32 neuronal islands reveal a higher number of cells identified in the traditional cultures (C), but an overall proportionately higher cellular density in islands (D). Functionally, activity rate, but not synchronization level, was elevated in islands compared to dissociated controls (E-F). Error bars indicate SEM.



Supplementary Figure 4: Representative examples of activity patterns in neuronal islands of varying cellular densities. High rates of uncorrelated activity (A) gradually transitioned to highly synchronous, low-rate activity (C) as cellular density was increased from low (<175 cells/mm²) to high (>250 cells/mm²) values.