Supra- and sub-threshold intracellular-like recording of 2D and 3D neuronal networks using nanopillar electrode arrays

Supplementary Information

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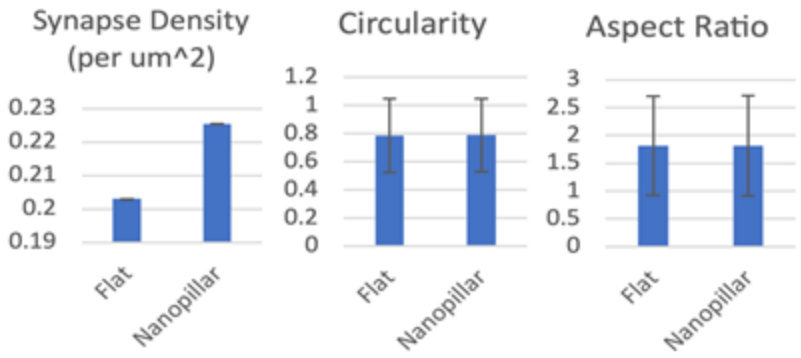

Figure S1. Summary of synapsin-1 metrics across flat and nanopillar surfaces.

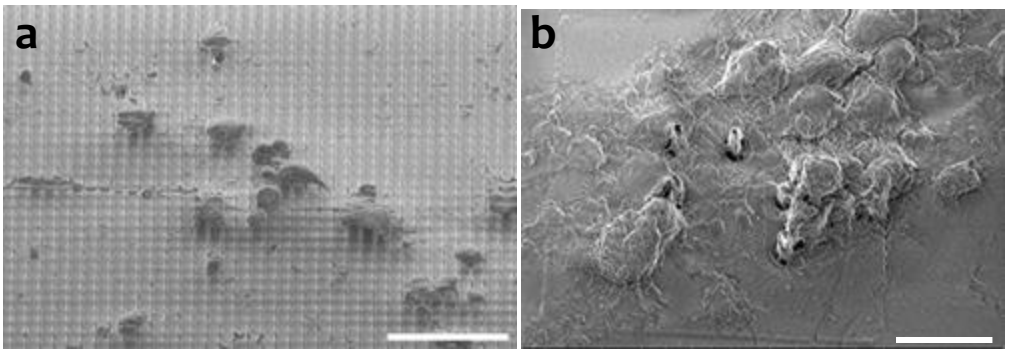

Figure S2. (a) Representative SEM image showing iPSC-derived neurons seeded on top of a “sea” of nanopillars, showing sparse interactions with iNeuron soma. Scale bar: 30 µm (b) Representative SEM image showing iNeurons interacting with a single nanoelectrode missing 3 pillars, showing sparse soma interactions. Scale bar: 10 µm.

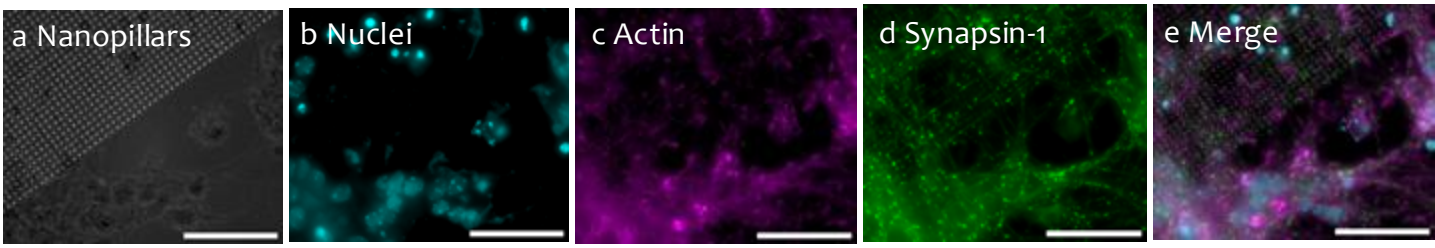

Figure S3. (a - e) Representative immunofluorescence images showing nanopillars, nuclei, actin, and synapsin-1 in separate channels (scale bars: 50 µm).

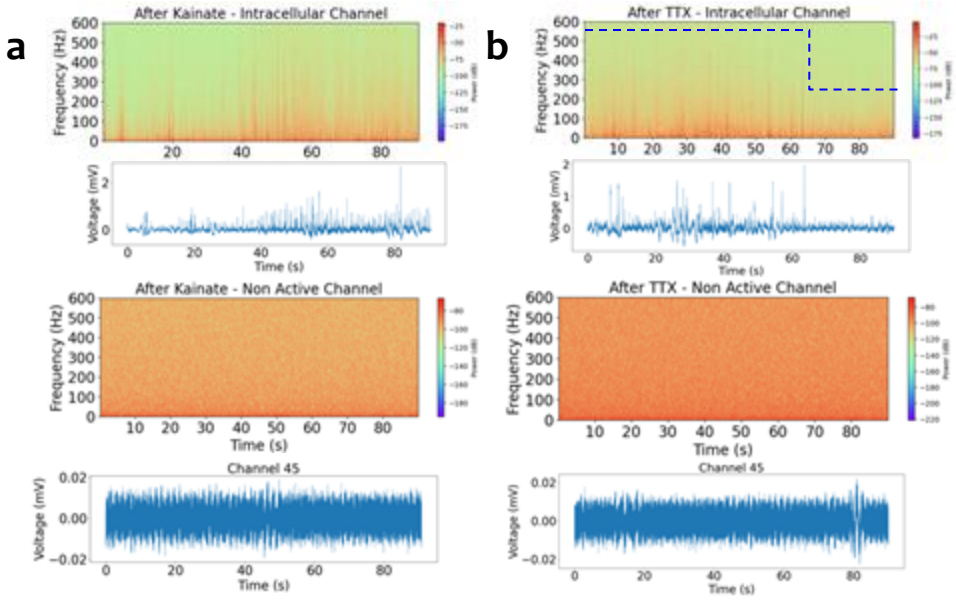

Figure S4. (a) Example intracellular and non-active (noisy) channels immediately after kainate addition, showing increased supra- (> 200 Hz) and sub- (<200 Hz) threshold activity only in the intracellular channel. Note that the downward spikes in the non-active channel represent 60 Hz electronic noise. (b) Example intracellular and non-active (noisy) channels immediately after tetrodotoxin addition, showing abolished supra- (> 200 Hz) and reduced sub- (<200 Hz) threshold activity only in the intracellular channel.

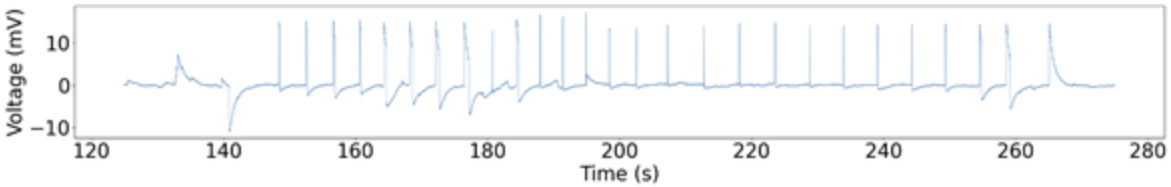

Figure S5. Recording trace showing “best” signals expected to have originated from a single neuron possessing tight coupling with a single nanoelectrode. The variations throughout the spike train suggest variable coupling over time, although the waveform shape, inter-spike interval, and frequency profiles suggest that these signals are biological in origin.

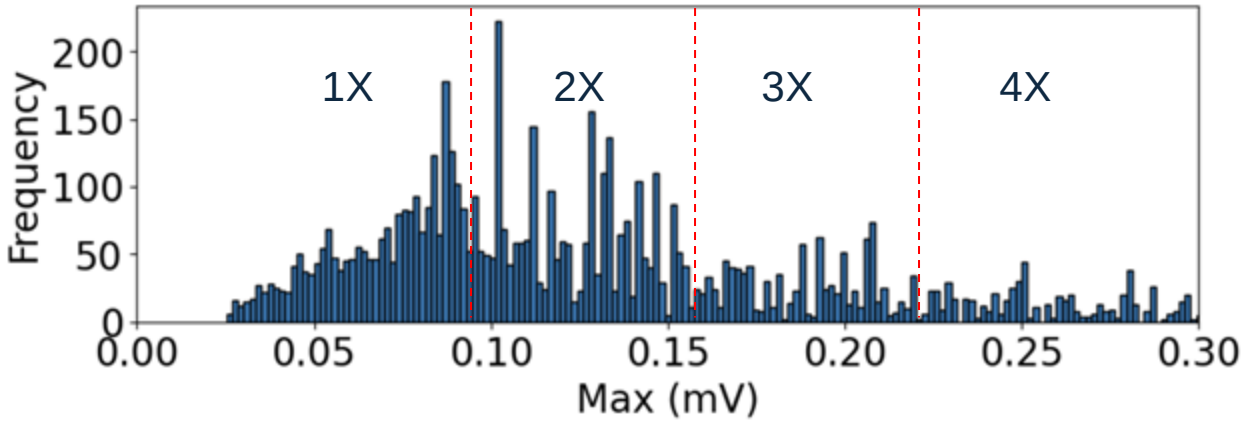

Figure S6. Histogram showing pooled data from all primary neuron spikes collected (N = 3270) across 4 different intracellular channels from one device during an entire recording session. This data was filtered above 70 Hz to eliminate any spikes from electronic noise, and spikes were detected using a threshold of 4x the standard deviation of the filtered signal. Metrics such as the maximum amplitude of the spike, the total amplitude, or even the minimum amplitude show similar “clustering” at rough multiples below 0.3 mV, the putative attenuated amplitude for subthreshold potentials.