## SUPPLEMENTARY INFORMATION

Pulse-width (ms)	Pulse Amplitude (µA)	Charge Density/Phase (mC/cm <sup>2</sup> )
0.1	1, 2, 5, 10, 20, 30, 40	0.02, 0.04, 0.10, 0.20, 0.41, 0.61, 0.81
0.5	1, 2, 3, 4, 5, 6, 7, 8	0.10, 0.20, 0.30, 0.41, 0.51, 0.61, 0.71, 0.81
1	1, 2, 3, 4	0.20, 0.41, 0.61, 0.81
2	1, 2	0.40, 0.81

**Table S1.** Parameters used for electrical stimulation of the retina. Charge densities are calculated for a  $25 \,\mu m$  diameter electrode.

Pulse-width (ms)	Pulse Amplitude (μΑ)	Charge Density/Phase (mC/cm <sup>2</sup> )
0.1	3, 6, 9, 12, 15, 18, 21, 24, 27, 30	0.06, 0.12, 0.18, 0.24, 0.31, 0.37, 0.43, 0.49, 0.55, 0.61
1	0.15, 0.25, 0.5, 0.75, 1, 2, 4, 6, 8, 10	0.03, 0.05, 0.10, 0.15, 0.20, 0.41, 0.82, 1.22, 1.63, 2.04
4	0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 4.5	0.20, 0.41, 0.82, 1.22, 1.63, 2.04, 2.45, 2.85, 3.26, 3.67

**Table S2.** Pulse width and range of amplitudes used in calcium imaging experiments. Charge densities are calculated for a 25  $\mu$ m diameter electrode.



**Figure S1. Equivalent circuit fitting for all diameters.** A subset of the EIS data from 25  $\mu$ m (n=25), 50 $\mu$ m (n=10) and 100 $\mu$ m (n=5) were used to assess the performance of our model. **(a)** Bode plot illustrating averaged measured (solid lines) and fitted (dashed lines) magnitude (left panel) and phase (right panel). **(b)** Boxplot of NRMSE values calculated from the fitting data shown in (a), for impedance fitting, NRMSE values were 0.095 ± 0.094 (25 $\mu$ m), 0.052 ± 0.006 (50 $\mu$ m), and 0.081 ± 0.016 (100 $\mu$ m); for phase fitting, NRMSE values were 0.085 ± 0.045 (25  $\mu$ m), 0.059 ± 0.005 (50  $\mu$ m), and 0.074 ± 0.009 (100  $\mu$ m). Statistical significance was assessed using the Mann-Whitney-Wilcoxon test, indicating no significant difference (n.s.).



**Figure S2. Schematic of the experimental setup.** The rGO MEA is positioned within a confocal imaging microscope for simultaneous fluorescence imaging and electrophysiological recordings. For hippocampal neuron cultures, spontaneous neural activity was recorded, followed by controlled electrical stimulation through the rGO MEA, capturing both neural spike data and calcium activity simultaneously. Retinal explants from Long-Evans rats are flat-mounted on the rGO MEA, with the ganglion cell layer facing the electrodes. The explants are subjected to full-field 450 nm LED light stimuli and biphasic current pulses of varying amplitudes and durations. Neural and calcium responses are recorded simultaneously, allowing for the comparison of light-evoked and electrically evoked activities. (Created with BioRender.com)



**Figure S3. Functional characterization of rGO MEAs on hippocampal cultures. (a)** Extended figure showing the network maturation over time. **(b)** Evolution of the electrical activity patterns recorded by electrodes with 25, 50 and 100μm diameter at DIV7 and DIV13. **(b)** Heatmap of the calculated SNR values superimposed on the MEA. Grey color depicts electrodes where spontaneous activity was not observed. **(d)** Modulation effects of varying the pulse width (stimulation & recording at the same electrode, 25μm). **(e)** Heatmap of the relative stimulation efficacy. Stimulating-electrode represented in red. As the applied voltage amplitude increases the region of response expands up to the full field of view (750 x 750 μm).



**Figure S4. Modulation of the spiking response in retinal explants. (a)** Spiking activity recorded by the highlighted electrode located in the inferior part of the MEA, capturing a single unit (unit 6), depicted in purple. The spiking response prior to L-AP4 perfusion (left), and cessation of the ON-type response following a 10-minute perfusion with 50  $\mu$ M L-AP4 (right). **(b)** Amplitude-dependent response dynamics for cathodic first charge balanced biphasic current stimulation (500  $\mu$ s pulse-width) recorded by an electrode 150  $\mu$ m apart from the stimulation electrode (size=25 $\mu$ m). Voltage traces from the last trial represented for each experiment. Orange window depicts the delivery of electrical stimulation.



**Figure S5. Description of the calcium imaging setup and results. (a) Top:** Schematics of the GCaMP protein functioning. Upon binding to Ca2+, GCaMP undergoes a conformational change that modulate its excitation/emission properties making it becomes brighter. **Bottom; Left:** Representation of the optical configuration of fluorescence imaging set up. The objective focuses the light for excitation and collects the fluorescence generated that is sent to the detector once it crosses through the transparent MEA substrate. **Right:** Image of the cells responding to electrical stimulation as highlighted by the change in fluorescence. Individual cell bodies can be distinguished. **(b)** Schematic of the imaging, recording and stimuli provision synchronization. **Right panel: (c)** Effect of pulse duration and amplitude in the spatial resolution of the GCL response. Stimulating electrode represented in red. Gray-outlined circles are GCaMP-expressing cells. The color-filled circles are cells responding to the stimuli. The color bar indicates the stimulation thresholds in terms of charge injection (mC/cm2) for each pulse duration. Scale bars: 50 μm. **(d)** Calcium traces elicited by 4 single charge-balanced biphasic current pulses of different pulse durations (0.1, 1, and 4 ms) applied every 10 s. In gray, the calcium traces of individual cells and in black the average cell response. The number of cells responding is indicated. **(e)** Boxplot representing the

distance of the activated cells from the edge of the active electrode (25 um diameter). Median () and mean (+) are indicated.



*Figure S6. Firing probability curves. Figure depicts the calculated firing probabilities for each pulse width. Green dots depict the firing probabilities at given current amplitudes, normalized by the peak spiking count. Purple dot represents the 50% firing probability where threshold current is defined. Orange line depicts the fitting by an exponential function.* 



**Figure S7. Electrical stimulation response histograms.** Plots depicting the histograms for different stimulation conditions. Kernel density functions were fitted over histograms in order to quantify the activation dynamics of the first 400 ms after current injection. Bottom right shows the fitted curves overlayed where areas under the curves were calculated. This allowed us to quantify the difference in activation dynamics with respect to the charge density achieved in different temporal windows.