Supporting Information

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SI Text

Plant Material and MEA Setup. Caryopses of Zea mays L. cv. Gritz (Maisadour semences, France) were soaked overnight in aerated tap water and placed between damp paper towels in Petri dishes. Dishes were maintained in vertical position, incubated at 26 °C for 48 h, and used after roots reached a length of ≈3 cm (usually after 2 days).

Longitudinal and transversal sections from the primary root tip were cut at 350 μm thickness and were stored submerged for 2 h in $CaCl_2$ 5 mM (pH 6.5) at room temperature (22 °C) before recording. For recording, slices were gently transferred on a multielectrode array (Fig. 1 *A* and *B*) with interelectrode distance of 500 μm or 200 μm and electrode diameter of 30 μm (Multi Channel System). The electrodes were coated with porous titanium nitride to minimize the impedance and to allows recording of APs at a high signal to noise ratio.

Photographs taken before and after recording sessions confirmed the localization of the recording electrodes. Recordings were performed with tissues submerged in bath solution containing $CaCl_2$ 5 mM (pH 6.5) at a flow rate of 1 mL·min^{-1} and temperature of 22 ± 1 °C.

To remove any organic contaminants on the surface, MEAs were cleaned with ethylic alcohol (70%) and then rinsed with demineralised water before any use.

Besides ensuring tissue vitality, the most critical aspect for successful recordings from slice preparations with MEAs is establishing and maintaining a very close and efficient contact between the tissue and the electrodes (36). In fact decreasing the electrode-tissue distance increases the seal resistance between the electrode tip and the reference electrode, consequently improving the signal-to-noise ratio. To achieve the best contact we fixed the slice onto the MEA using an adhesive water permeable and water resistant tape (3M Micropore Surgical Tape) that mediate close and flat adhesion of the slice to the MEA surface, allowing in the meantime superfusion of the tissue. Cell death in root slices was routinely assessed at the end of each measurement using the fluorescent exclusion dye propidium iodide (PI) (Molecular ProbesA)

Membrane Potential Measurements. Conventional KCl-filled Ag/AgCl microelectrodes with tip diameter ≈0.5 μm were used to measure the membrane potential of cortical cells in intact roots.

Microscopy. Confocal laser scanning microscopy was performed using an upright Leica SP5, and a 20× objective. The images were captured with the LAS AF image acquisition software (Leica). Photoshop imaging suite was used for further image handling.

Pharmacology. For pharmacological experiments, root apices where submerged into appropriate solutions at room temperature. Spontaneous activity was increased by bath perfusion with Lglutamate (Glu) and inhibited by the glutamate receptor antagonist 6,7-Dinitroquinoxaline-2,4-dione (DNQX, 0.4 mM, pH 6.7, 2-h incubation) (44) dissolved in 2.5% DMSO or the Ca^{2+} channel blocker gadolinium chloride (Gd^{3+}, 1 mM, 2-h incubation). Glu was obtained from VWR, Gd^{3+} from Merck Pty. Limited, $CaCl_2$ from JT Baker Chemical Co., all of the other chemicals were obtained from Sigma chemicals. Each solution was adjusted to pH 6.7 and used, without root sample, as negative control to check electrodes signal-to-noise ratio.

Data Acquisition. After positioning the sliced tissue, and checking its correct position by inverted microscope, the MEA was connected to a 60-channel amplifier used at a 1200× amplification (MEA1060BC, Multi Channel System). Data were acquired by a data acquisition card (16 bit, MC card, Multi Channel System) at a sampling rate of 20 KHz. Dedicated software such as MC Rack (Multi Channel Systems) was used to control the hardware and record simultaneously field potentials from the 60 electrodes.

Signal Processing. Negative spikes were detected by setting the threshold at −30 μV (≈5 times the RMS, root mean square, value). Raw data and spikes waveform were stored for off-line analysis. IDL-based software was used for spike sorting and train analysis.

The rate of the signals recorded was calculated as:

$$R(t)\frac{1}{M}\sum_{j=1}^{M}\left[\frac{1}{Me}\sum_{i=1}^{Me}\frac{Ni}{(t+\Delta t)}\right]_{\mathrm{j}}$$

where M is the number of experiments; Me the number of active electrodes; Ni the number of events and Δt the time unit (10 s).

To visualize the spatial localization and spread of the signals, we generated color-coded activity maps representing the amplitude or rate of the spikes recorded by each MEA electrode using cubing interpolation for amplitude ratios at intermediate positions.

To detect spike coincidences (42, 43) between the spike trains measured at different groups of electrodes A and B, we use the coincidence probability defined as follows:

$$P(\tau) = N[(A, B_\tau)/(N(A) + N(B_\tau)]$$

where time τ is a temporal shift taking the values: $\tau = \pm n\Delta$t for n being a natural number and Δt a temporal bin of size Δt. $N(A)$ represents the number of events detected in each temporal bin Δt throughout the overall spike train at electrodes A, and $N(B_\tau)$ is the number of APs detected in each temporal bin Δt throughout the shifted in time by τ AP_S train at electrodes B.

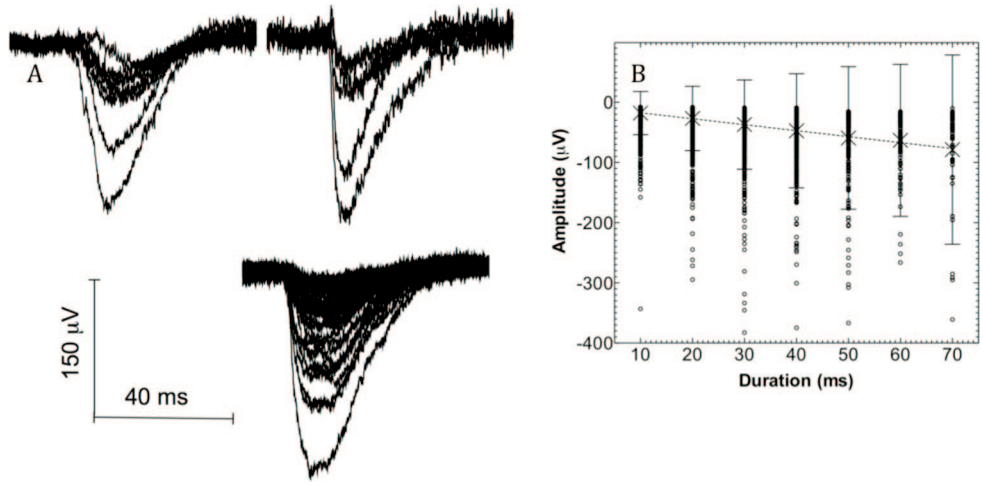
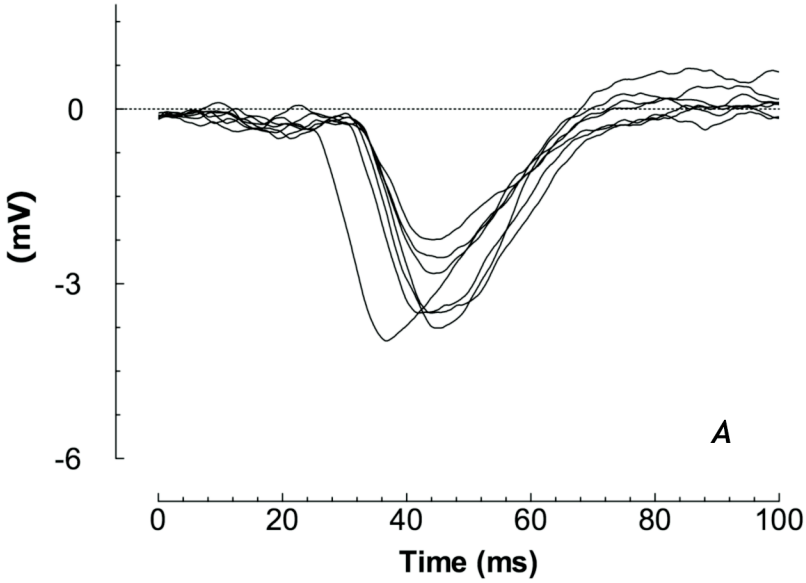


Fig. S1. (*A*) Overplots of spikes recorded with the MEA system in maize root in 3 different experiments. (*B*) Amplitude plotted against duration of action potentials. Black points, amplitude and duration for each spike; X, average values ± SD; dotted line, linear fit for averaged values.



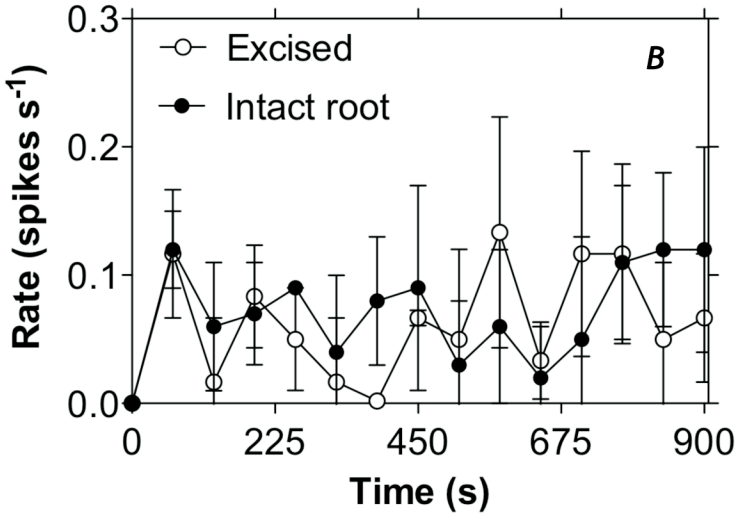


Fig. S2. (*A*) Overplots of spikes recorded intracellularly with conventional microelectrodes in 3 intact root apex of maize. (*B*) Comparison of the rate of the spontaneous activity recorded with MEAs in excised roots and with conventional microelectrodes in intact roots. To make the data comparable, just cells impaled in the same regions of that considered with the MEA, that showed spontaneous activity were considered.

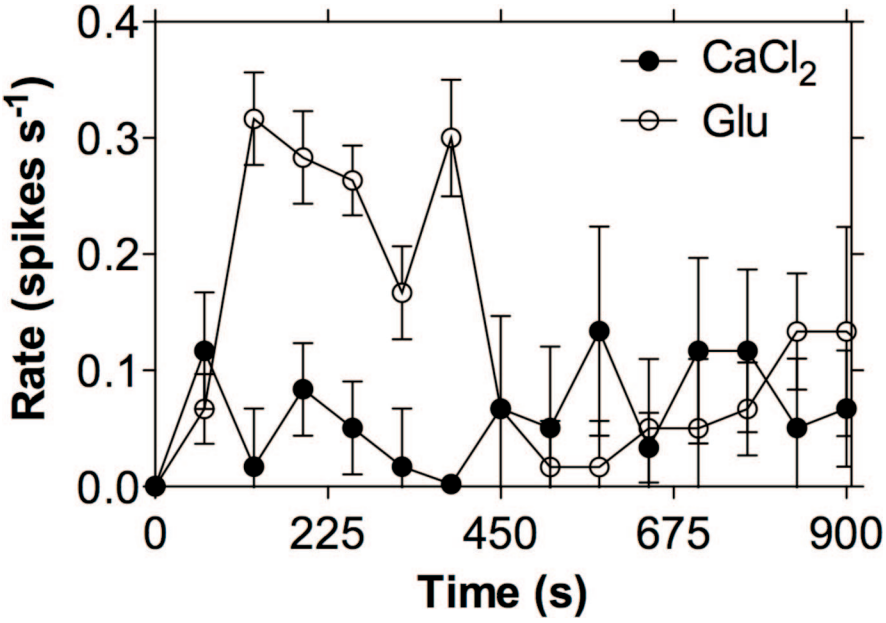


Fig. S3. Rate of the spontaneous activity recorded in maize root cells under different condition: $CaCl_2$ 5 mM and $CaCl_2$ 5 mM + Glu 1 mM. Data are presented as means ± SD from 7 different experiments.

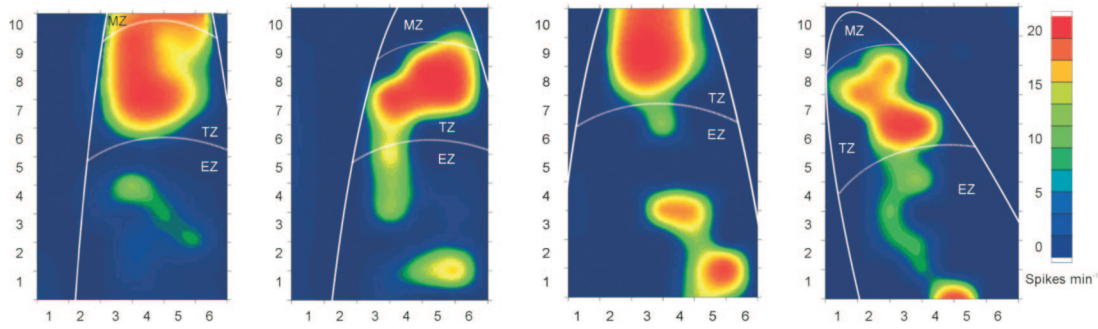
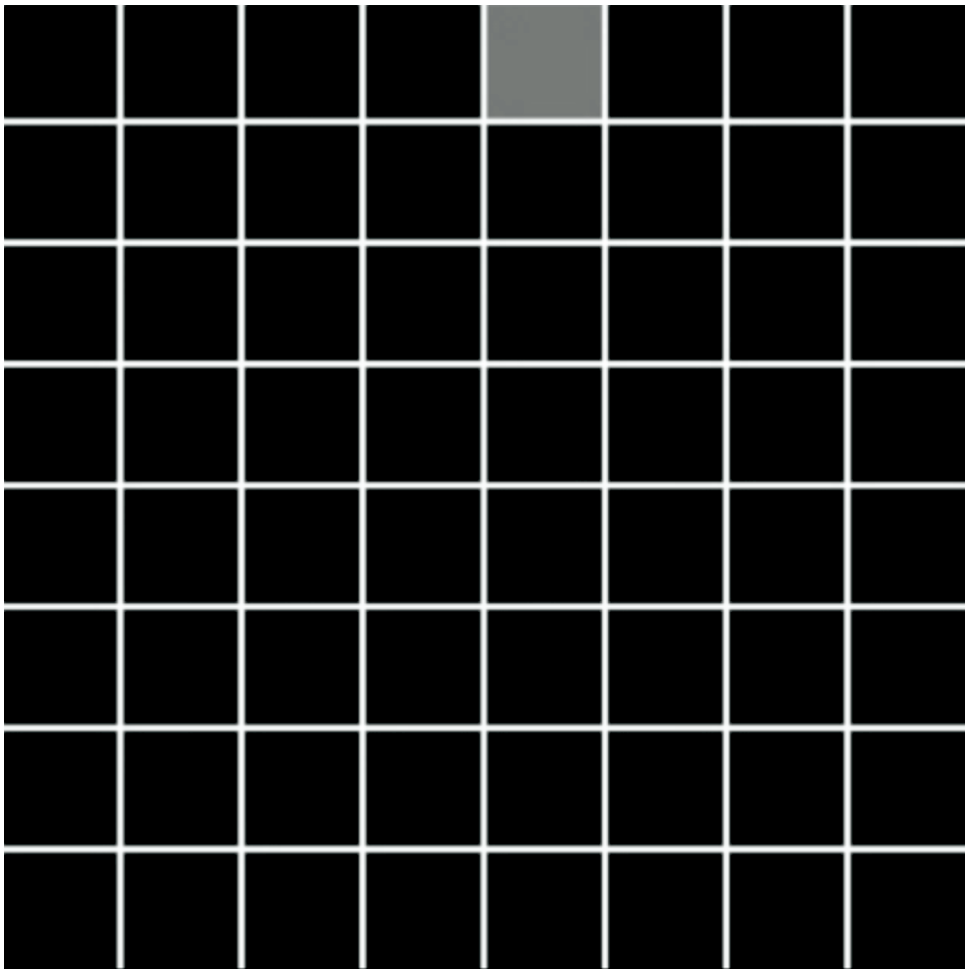


Fig. S4. Representative images of the spatial localization of the electrical activity in root apex of maize. Overall electrical activity measured as rate of appearance (spikes per minute) in 4 different roots/experiments. Root tips were always positioned in the upper part of the MEA array.



Movie S1. Electrical activity in the root apex.

Movie S1 (AVI)