Supplementary Information

Supplemental Note 1. Reconstruction of the intracellular potential

As shown in Fig. 3B, the signals recorded by the chip are a distorted version of the actual signals (recorded by patch clamp). This can be attributed to the presence of a reference voltage compensation circuit (DC compensation loop) in the input amplifier (10,18), which eliminates electrode drift and offset effects. However, for low-frequency signals, this compensation acts as a high-pass filter and generates distortion. The amplifier transfer function can be modeled as:

$$H_{amp} = A_1 \frac{1+sC_fR_f}{A_2R_fg_{mf}+sC_fR_f}$$  \[1\]

where $A_1$ is the amplifier gain, $A_2R_fg_{mf}$ is the feedback gain, and $C_fR_f$ is the feedback loop time constant. All these parameters are determined by an automated calibration phase before the measurement. By applying the inverse of this transfer function to the measured signals, a reconstruction of the intracellular potential can be obtained:

$$V_{m,\text{recon}}(t) - V_{\text{rest}} \approx V_o(t) * \mathcal{L}^{-1} \left\{ \frac{1}{A_1} \cdot \frac{A_2R_fg_{mf} + sC_fR_f}{1+sC_fR_f} \right\}$$  \[2\]

where $\mathcal{L}^{-1}$ represents the inverse Laplace operator. The term $V_{\text{rest}}$ represents the membrane resting potential. As the DC signal information was lost due to the capacitive coupling at the electrode, this term was subtracted from the reconstructed
signal. The inverse filtering was applied to the raw data using Matlab (The
Mathworks).

**Supplemental Note 2. Model derivations**

Given the linear circuit model of Fig. 3A, the transfer function (in the Laplace
domain) from the intracellular potential $V_m$ to the electrode potential $V_e$ can be
expressed as:

$$
\frac{V_e(s)}{V_1} = \frac{R_{seal}(1+sC_{m1}R_p)(1+sC_sR_s)}{R_p + R_{seal} + s[C_L(R_pR_s + R_pR_{seal} + R_sR_{seal}) + C_{m1}R_pR_{seal} + C_sR_s(R_p + R_{seal})] + s^2R_pR_sR_{seal}[C_{m1}(C_L + C_s) + C_LC_s]} \tag{3}
$$

where $R_{seal}$ represents the ionic cleft formed between the plasma membrane and the
chip surface and has typical values between 1-10 MΩ (12,13); $C_{m1}$ represents the
capacitance of the fraction of the cell membrane located on top of the electrode and
can be approximated by $C_{m1} = A_s c_m \approx 100 fF$, where $A_s$ is the electrode area (i.e. 5
µm²) and $c_m$ is the characteristic capacitance of the cell membrane (i.e. ~20 fF/µm²);
$R_p$ represents the resistance of the pore being generated by the electroporation pulses;
$C_s$ is the linearized model of the electrode double layer capacitance; $R_s$ represents the
Faradaic charge transfer at the electrode interface. $C_L$ is the representation of the input
capacitance of the amplifier located beneath the electrode which was approximately
300 fF. Impedance measurements of the electrode resulted in values of $C_s \approx 30 pF$
for electrode areas of 5 µm² and $R_s > 10 GΩ$. Such high characteristic capacitance
was obtained due to a 300 nm thick TiN layer on top of the electrodes. Given the
following assumptions: $C_s \gg C_L$, $R_s \gg R_{seal}$ and $C_s \gg C_{m1}$, we can approximate Eq. 3 as:

$$\frac{V_e}{V_i}(s) \approx \frac{\alpha \cdot (1+s \cdot C_{m1} R_p)(1+s \cdot C_s R_s)}{1+s \cdot C_s R_s + s^2 \cdot \alpha R_p R_s C_s (C_{m1} + C_L)}$$  \hspace{1cm} [4]

where $\alpha = R_{seal}/(R_p + R_{seal})$. High values of $\alpha$ can be achieved for high values of $R_{seal}$ which can only be obtained if the cell fully covers the electrode (5,6).

When the term $(2\pi C_s R_s)^{-1}$ is lower than the minimum signal frequency of interest, a condition that is met with a large enough electrode capacitance, we can approximate Eq. 4 as:

$$\frac{V_e}{V_i}(s) \approx \frac{\alpha \cdot (1+s \cdot C_{m1} R_p)}{1+s \cdot \alpha R_p (C_{m1} + C_L)}$$  \hspace{1cm} [5]

Similarly, if the maximum signal frequency of interest is lower than the term $(2\pi C_{m1} R_p)^{-1}$, the transfer function in Eq. 3 can be further reduced to:

$$\frac{V_e}{V_i}(s) \approx \alpha = \frac{R_{seal}}{R_p + R_{seal}}$$  \hspace{1cm} [6]

Since most physiological signals are within a 2 kHz bandwidth, previous condition can be re-written as: $\pi^2 d_s^2 c_m (2g_p)^{-1} > 2 \text{kHz}$, where $g_p$ is the pore conductivity and $d_s$ is the electrode diameter.
Supplemental Table

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<th>Stim. Voltage (V_p-p)</th>
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<th>Pulse Width (ms)</th>
<th>ISI Before (s)</th>
<th>Stdev Before (s)</th>
<th>ISI During (s)</th>
<th>Stdev During (s)</th>
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<th>Stdev After (s)</th>
<th>p Value</th>
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</table>

ISI is the interspike interval calculated using a peak detection algorithm. The p-values were calculated using the non-parametric Friedman’s test for comparison of three different groups (Matlab, The Mathworks; GraphPad).

Supplemental Figure S3

Fig. S3. Prolonged open-cell state by applying consecutive stimulation pulses on the same cell. Blank spaces between open-cell recordings mark electroporating stimuli.