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SUPPLEMENTARY INFORMATION

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Vertical nanowire electrode arrays as a scalable platform for intracellular interfacing to neuronal circuits

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Device Fabrication

Vertical nanowire electrode arrays (VNEAs) were fabricated on a silicon-on-insulator (SOI) wafer with a degenerately doped device layer (Ultrasil Corp., *n*- and *p*-type, 0.001 – 0.005 Ohm·cm), as depicted in Supplementary Fig. 1. Our prototype VNEA device consisted of 16 stimulation/recording pads (200 µm pitch), each containing 9 vertical silicon nanowires (3 × 3, 2-µm pitch, Figs. 2A-C). Silicon nanowires (~ 150 nm in diameter and 3 µm in length) were fabricated via reactive ion etching (RIE) and thermal oxide thinning. Briefly, 600-nm diameter holes in poly(methyl methacrylate) were patterned via electron-beam lithography. Subsequent evaporation and liftoff yielded 30-nm thick alumina discs that served as an etch mask. Reactive ion etching of the surrounding silicon surface produced 3-µm tall silicon nanowires that were then thinned down via thermal oxidation and wet chemical etching. The silicon surface was next passivated with thermally grown SiO_2 (20 ~ 40 nm in thickness). Selective metallization of the

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nanowire tips was achieved by spinning a thin layer of photoresist (500 nm, Shipley 1805) so as to leave the nanowires' tips exposed. After the tips' oxide layer was stripped with buffered HF, the exposed silicon was metalized with 10 nm of Ti followed by 50 nm of Au via sputtering and liftoff. To improve contact resistance, the VNEAs were annealed at 250^{o}C in nitrogen. Subsequently, stimulation/recording pads were made independently addressable by first defining electrode tracks using photolithography and then etching away the interstitial regions via RIE. These tracks were then passivated via atomic layer deposition of a 100-nm layer of Al_2O_3. The Al_2O_3 layer covering the nanowires was removed via photolithography and selective wet chemical etching (TransEtch-N, Transene). Finally, a metal contact pad (50 nm Ti/50 nm Au) was added to each electrode track to facilitate electrical interfacing to our measurement setup.

Cell Culture

Dissociated E18 rat cortical neurons (BrainBits LLC) were cultured on top of VNEAs as previously described[1]. A three-well polydimethylsiloxane (PDMS) washer structure was used: a central double well (diameter: 3 mm, each) was employed to retain a droplet of cells above the VNEA during the initial 45 minutes needed for cell settling[1]. Two additional wells (diameter: 5 mm) were also seeded with cells so as to help support neuronal viability by conditioning the media within the larger washer structure[2] (Supplementary Fig. 1b). Prior to the addition of cells, exposed device surfaces were coated with poly-L-lysine (typically 2 – 5 nm in thickness) to promote cell adhesion and growth.

Equivalent circuit analysis of the VNEA-cell interface

Values of the circuit elements shown in Fig. 2A were determined using the input impedances of the patch pipette and the VNEA pads, as well as the electrical coupling between the two. After forming a seal with the patch pipette (prior to membrane rupture), the pipette seal resistances ($R_{s,p}$) were measured to be >2 GΩ based upon the steady state current resulting from 10 mV test pulses applied to the patch pipette. The pipette access resistance ($R_{a,p}$), membrane resistance (R_m) and membrane capacitance (C_m) were then measured from transient current responses to 10 mV test pulses after rupture using the pCLAMP 10 software (Molecular Devices) and Matlab. Values of $R_{a,p}$ were typically between 15 and 30 MΩ, within the normal range for the 5 ~ 10-MΩ patch pipettes used in our experiments. Because this value was small compared to $R_{s,p}$, currents through the pipette seal were neglected from the subsequent analysis. Typical R_m and C_m values for HEK293 cells were between 150 ~ 600 MΩ and 10 ~ 30 pF respectively, consistent with those reported in literature[22].

After obtaining a whole cell patch with a pipette, current injected through the pipette (in the current-clamp mode) and the VNEA were used to determine the values of the remaining circuit elements. The nanowire seal resistance ($R_{s,NW}$) was determined to be 100 ~ 500 MΩ based on the change ($\Delta R_{in,p}$) in the access-adjusted pipette input impedance ($R_{in,p} \equiv [1/R_m + 1/R_{s,NW}]^{-1}$) before ($R_{in,p0}$) and after ($R_{in,p1}$) membrane permeabilization at the nanowire-cell junction (Supplementary Fig. 4):

$$R_{s,NW} = \frac{R_{in,p0} R_{in,p1}}{\Delta R_{in,p}}.$$

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The ratio (κ_p) between the pipette voltage change (ΔV_p) and the nanowire voltage change (ΔV_{NW}) during nanowire current injection can be related to the nanowire access resistance ($R_{a,NW}$) and the leak-adjusted nanowire input impedance ($R_{in,NW} \equiv R_{NW} + [1/R_m + 1/R_{s,NW}]^{-1}$) by:

$$\kappa_p = \frac{\Delta V_p}{\Delta V_{NW}} = \frac{R_{in,NW}}{R_{in,NW} + R_{a,NW}}.$$

Note that similar to the pipette access resistance, $R_{a,NW}$ includes the intrinsic nanowire electrode resistance, the resistance at the electrochemical junction, and the resistance through the permeabilized membrane interface.

Meanwhile, the ratio (κ_{NW}) between ΔV_{NW} and ΔV_p during current injection via the pipette can be related to $R_{a,NW}$ and the VNEA leak resistance R_L (either through defects in electrode insulation or uncoupled nanowires) by:

$$\kappa_{NW} = \frac{\Delta V_{NW}}{\Delta V_p} = \frac{R_L}{R_L + R_{a,NW}}.$$

Typical values for κ_{NW} and κ_p in our experiments were 0.2 ~ 0.5 and 0.04 ~ 0.1, respectively. From these measured values, we can calculate the $R_{a,NW}$ and the R_L:

$$R_{a,NW} = \frac{R_m R_{s,NW}}{R_m + R_{s,NW}}\left(\kappa_p^{-1} - 1\right),$$

$$R_L = \frac{R_{a,NW}}{\kappa_{NW}^{-1} - 1}.$$

$R_{a,NW}$ and R_L typically ranged between 0.3 and 1.5 GΩ, consistent with the total nanowire input impedance $R_t \equiv R_{NW} + [1/R_L + 1/R_{in,NW}]^{-1}$ measured experimentally. Note that due to electrochemical reactions at the nanowire tip, $R_{a,NW}$ is a nonlinear function of V_{NW}, and the value presented above represents those determined at a typical operating voltage of -1.5 V with respect to the bath electrode.

RC time constants of the VNEA measurement setup.

Bare nanowire RC time constants were measured as follows. At the conclusion of a recording session, the patched cell was removed from the recording pad by slowly withdrawing the patch pipette. The current flowing through the nanowires was then increased by 200 pA for 300 ms and the corresponding voltage change was recorded. The transient portion of the response could be well described by a single exponential with a time constant between 5 and 50 ms. We determined the nanowire resistance from the steady-state potential difference at the two nanowire currents. Dividing the exponential time constant by this resistance yielded the value of the parasitic capacitance (C_p), which was typically 150 pF. We determined the parasitic capacitance of our current-clamp electronics to be 50 pF by applying the same protocol using a 1 GΩ load resistor.

Using the measured RC time constant of a VNEA pad, we could deconvolve voltage waveforms measured by the VNEA pad and recover true membrane potential waveforms. As an example, we used a model action potential (AP) waveform to control the membrane potential of a HEK293 cell. The time-averaged VNEA recording is shown in Supplementary Fig. 5. To recover the original waveform, we rescaled the measured signal according to the measured coupling constant (κ_{NW}) and performed a Weiner deconvolution in Matlab using the measured time constant of 9.3 ms. The deconvolved VNEA measurements showed good agreement with the original waveform applied to the pipette (Supplementary Fig. 5).

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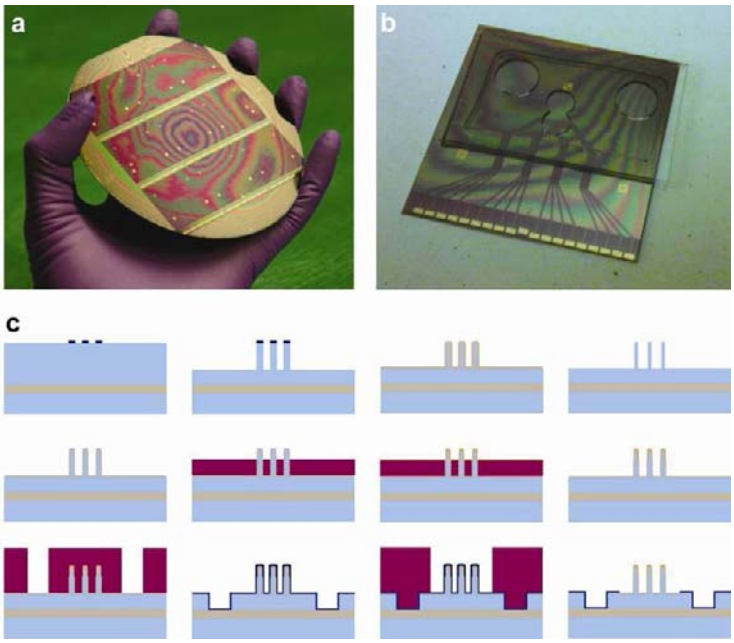
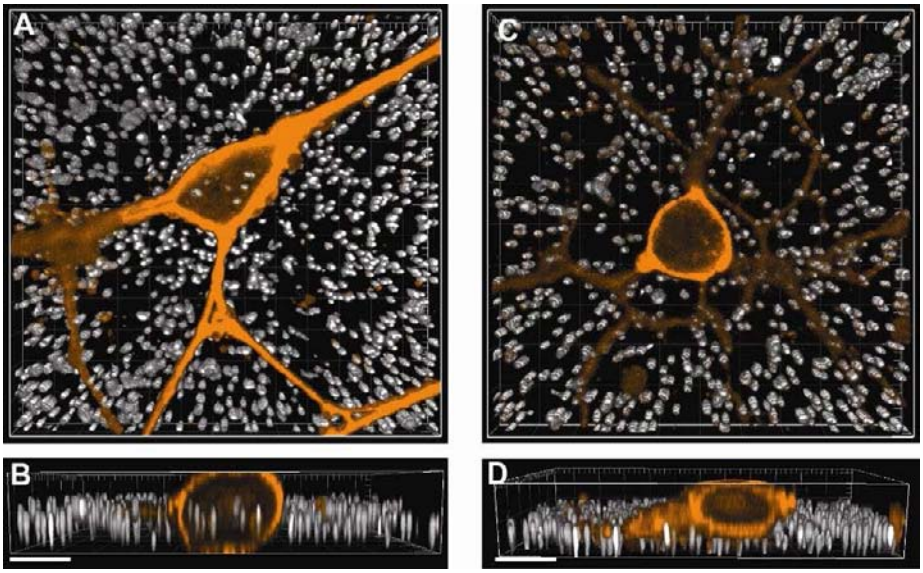
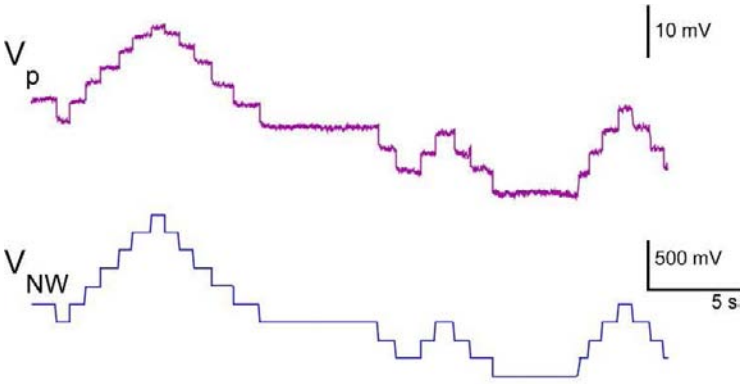
Supplementary Figures:



Supplementary Figure 1. VNEA device fabrication. (**a**) Image of a set of 9 completed VNEA devices, fabricated in parallel on a 4-inch SOI wafer. (**b**) Image of a representative VNEA device consisting of 16 stimulation/recording pads and a polydimethylsiloxane (PDMS) washer with three cell-culture wells: a central double well that surrounds the active region of the device and two additional wells that hold neurons for media conditioning. (**c**) Device fabrication process flow. Briefly, from left to right, then top to bottom: devices were fabricated on an SOI wafer with degenerately doped silicon device and handle layers (cyan) and a 2 – 4 μm thick buried oxide layer (gray). Electron-beam lithography, followed by aluminum evaporation and liftoff, yielded 600-nm diameter aluminum discs (blue) at positions intended for nanowire fabrication. The aluminum discs served as a hard mask for RIE, yielding arrays of silicon nanowires. The nanowires were then thinned down via thermal oxidation and wet chemical etching to obtain diameters of 150 nm. Next, SiO_2 (gray, 20 - 40 nm in thickness) was grown thermally, and a thin layer of photoresist (red) was spun on top, leaving the nanowire tips exposed. Oxygen plasma cleaning and buffered oxide etch were used to remove the SiO_2 covering the nanowire tips. Immediately following oxide removal, the sample was sputter-coated with metal (yellow). Subsequent liftoff yielded metal caps only at the nanowire tips. Next, photoresist was patterned to define the electrode tracks used to address the nanowires, and the interstitial silicon was etched away using RIE. The entire device was then insulated using an Al_2O_3 layer (blue). The Al_2O_3 layer covering the nanowires was then removed using selective wet chemical etching followed by removal of the photoresist.



Supplementary Figure 2. Nanowire penetration of neuronal cell membranes. Three-dimensional confocal reconstructions show that the membranes of some neurons (orange) appear penetrated by nanowires (white) (**A, B**), while others appear to rest on top of the nanowires (**C, D**).



Supplementary Figure 3. Steady state control of the membrane potential. The membrane potential in a 0DIV HEK293 cell recorded via patch pipette (V_p, magenta) can be controlled by changing the offset voltage applied to the nanowire electrode (V_{NW}, blue)

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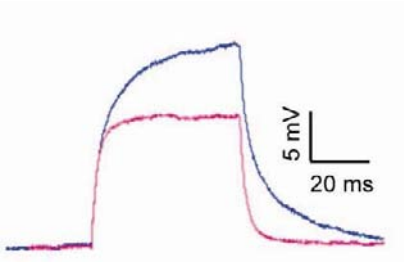
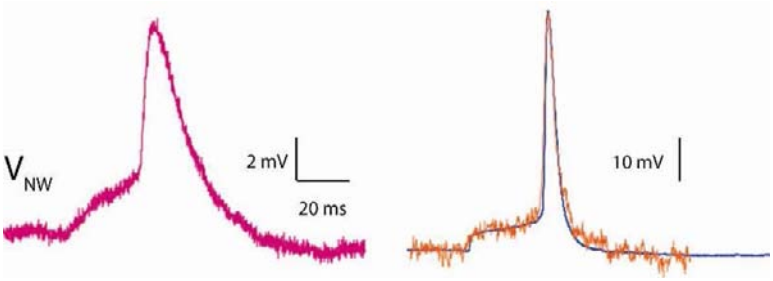
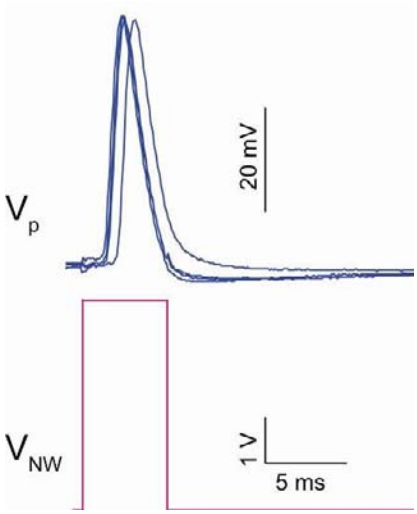


Supplementary Figure 4. Membrane characteristics before and after permeabilization. Typical voltage response to 50-pA current pulses before (blue) and after (magenta) membrane permeabilization at the nanowire-cell junction. Measurement and stimulation were performed on a HEK293 cell (0 DIV) using a patch pipette in whole-cell current-clamp mode.



Supplementary Figure 5. Deconvolution of VNEA measurements. A model AP waveform generated via a patch pipette is measured using the VNEA in current-clamp mode (magenta). Deconvolution of the VNEA measurement (orange) shows good agreement with the waveform applied at the patch pipette (blue). Plots are averaged over 5 recordings.



Supplementary Figure 6. Stimulation of neuronal action potentials using the VNEA. Neuronal APs (measured via a patch pipette, blue) were reliably evoked by applying voltage pulses to the VNEA. Time-aligned overlay of 5 consecutively stimulated APs show < 1 ms jitter.

References:

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