

High Density Individually Addressable Nanowire Arrays Record Intracellular Activity from Primary Rodent and Human Stem Cell Derived Neurons

Ren Liu,[†] Renjie Chen,[†][©] Ahmed T. Elthakeb,[†] Sang Heon Lee,[†] Sandy Hinckley,[¶] Massoud L. Khraiche,[†] John Scott,[‡] Deborah Pre,[¶] Yoontae Hwang,[†] Atsunori Tanaka,[§] Yun Goo Ro,[†] Albert K. Matsushita,[§] Xing Dai,^{†,#} Cesare Soci,[#] Steven Biesmans,[¶] Anthony James,[∥] John Nogan,[∥] Katherine L. Jungjohann,[∥] Douglas V. Pete,[∥] Denise B. Webb,[∥] Yimin Zou,[‡] Anne G. Bang,[¶] and Shadi A. Dayeh^{*,†,§,⊥}[©]

[†]Integrated Electronics and Biointerfaces Laboratory, Department of Electrical and Computer Engineering, [‡]Neurobiology Section, Biological Sciences Division, [§]Graduate Program of Materials Science and Engineering, and [⊥]Department of NanoEngineering, University of California San Diego, 9500 Gilman Drive, La Jolla, California 92093, United States

[¶]Conrad Prebys Center for Chemical Genomics, Sanford Burnham Prebys Medical Discovery Institute, 10901 North Torrey Pines Road, La Jolla, California 92037, United States

[#]Division of Physics and Applied Physics, Nanyang Technological University, 21 Nanyang Link, Singapore 637371, Singapore

^{||}Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, New Mexico 87185, United States

Supporting Information

ABSTRACT: We report a new hybrid integration scheme that offers for the first time a nanowire-on-lead approach, which enables independent electrical addressability, is scalable, and has superior spatial resolution in vertical nanowire arrays. The fabrication of these nanowire arrays is demonstrated to be scalable down to submicrometer site-to-site spacing and can be combined with standard integrated circuit fabrication technologies. We utilize these arrays to perform electrophysiological recordings from mouse and rat primary neurons and human induced pluripotent stem cell (hiPSC)-derived neurons, which revealed high signal-to-noise ratios and sensitivity to subthreshold postsynaptic potentials (PSPs).



We measured electrical activity from rodent neurons from 8 days in vitro (DIV) to 14 DIV and from hiPSC-derived neurons at 6 weeks in vitro post culture with signal amplitudes up to 99 mV. Overall, our platform paves the way for longitudinal electrophysiological experiments on synaptic activity in human iPSC based disease models of neuronal networks, critical for understanding the mechanisms of neurological diseases and for developing drugs to treat them.

KEYWORDS: Nanowire, neuron, intracellular, extracellular, subthreshold, drug-screening

 \mathbf{N} anowire geometries are ideal for interfacing with cells and measuring intracellular potentials of neurons with minimal invasiveness.¹⁻⁶ Prior works have demonstrated single nanowire devices^{7,8} or devices encompassing ensembles of several nanowires,^{9–13} but individual electrical addressability of a single nanowire in a vertically standing array of nanowires, which is important for localizing the origin of action potentials, has not been accomplished before. Additionally, interfacing with human neurons, important for drug screening, is yet to be demonstrated. Here, we report a new hybrid integration scheme that offers for the first time a nanowire-on-lead approach, which enables independent electrical addressability, is scalable, has a high spatial resolution, and has a sensitivity that allows the detection of subthreshold PSPs. The fabrication of these nanowire arrays is demonstrated to be scalable down to submicrometer site-to-site spacing and can be combined with standard integrated circuit fabrication technologies. Physiological recordings from rodent primary neurons and human induced pluripotent stem cell (hiPSC)-derived neurons revealed high signal-to-noise ratios (SNRs) for both positive and negative measured potentials. We measured electrical activity from rodent neurons from 8 DIV to 14 DIV, and from hiPSC-derived neurons at 6 weeks in vitro post culture, and found intimate nanowire/neuron interaction validated by transmission electron microscopy (TEM). The technique contrasts to the standard patch-clamp,^{14–17} which is destructive and unscalable to large neuronal densities and to long recording times,¹⁸ or to planar multielectrode arrays that enable long-

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Figure 1. (A) Illustration of fabrication procedure for high density electrically isolated nanowire probes by solid-state wafer bonding. (i) Metal stack with Ni topmost layer is patterned by a combination of photolithography and electron beam lithography atop an electrically insulating and transparent sapphire substrate. (ii) Si is bonded to the substrate in i by nickel silicidation. (iii) Si wafer is thinned down to the desired wire height. Ni masks are then defined by electron beam lithography and aligned to the bottom plane Ni pattern. (iv) Si nanowires are etched by an SF₆/C₄F₈ plasma etch step. (v) SiO₂ PECVD is then deposited and is selectively etched to expose the tips of the Si nanowires. (B, C) SEM images of an 8×8 Si nanowire array (B) after etching and (C) after SiO₂ passivation. Scale bar in B is 5 μ m and in C is 3 μ m. (D, E). Energy-dispersive X-ray spectroscopy (EDX) of the (D) oxygen signature of the SiO₂ passivation layer, (E) nickel for etching mask (top), NiSi region, and conducting lower most layer, and (F) Ti as the diffusion barrier (top) and adhesion layer (bottom). Scale bars are 1 μ m. (G) High magnification TEM image of the NiSi/Ti/Ni/Ti underneath the Si nanowire highlighting the interfacial structure between the bottom conducting lead and the Si nanowire. Scale bar is 200 nm. The bottom panels are HRTEM images at the interface between Si and NiSi with electron beam axis aligned in the Si [T10] (left panel), and the NiSi [T10] Zone axis in (right panel) to display the crystalline interface. Scale bars in bottom panels are 2 nm.

term recordings, but can just measure extracellular potentials and lack the sensitivity to subthreshold potentials.¹⁹

To measure minute potential changes in individual cells at high spatial resolution in neuronal networks, it is important to develop an integration scheme for high-density individually electrically addressable out-of-plane nanowire neuronal probes. To achieve very high densities of individual nanowires that are suitable for mapping individual units in neuronal networks, we devised a novel all solid-state wafer bonding integration scheme on patterned Ni electrical contacts and leads (Figure 1, Figures S1 and S2)²⁰ leading to a superior high-density nanowireneuron interface platform. This bonding scheme is essential because conventional low temperature eutectic bonding does not provide the lead-to-lead electrical isolation necessary for individual electrical addressability of single sites in a highdensity nanowire array.²¹ Instead, we utilize the thermally driven solid-state diffusion of Ni into Si at a low temperature (400 °C), traditionally used to make self-aligned contacts for transistors in the semiconductor industry,²² to bond Si substrates to optically transparent and electrically insulating sapphire substrates that were predefined with Ni patterns. By doing so, we achieve two goals with the Ni layer: (1) bonding and fusion of a thin (~50 μ m) Si substrate to the underlying host substrate and (2) embedding electrical leads underneath active or passive Si components in the bonded substrate with low contact resistance (Figure S1). The integration technique is

general to any other substrate that can sustain the NiSi reaction temperature (starts at 300 °C), including complementary metal oxide semiconductor (CMOS) integrated circuits and advanced $\mathsf{planar}^{23,24}$ and out of plane device geometries are attainable through this method. Additionally, the optical transparency of sapphire enables light excitation and transmission imaging degrees of freedom in our platform. Overall, the integration technology developed in this work is the first to enable electrical addressability for individual vertically standing nanowires registered precisely over underlying metal leads. This individual electrical addressability of nanowires has potential to enable precise measurements of activity of individual units in neuronal networks, and to detect miniature release of neurotransmitters, especially important for investigating the synaptic properties of networks of neurons in the context of neurological diseases, as for the characterization of pre- or post- synaptic defects based on amplitude or frequency modifications in the subthreshold postsynaptic potentials.^{25,26}

Fabrication of our in vitro platform starts with photolithography and e-beam lithography (EBL) patterning of electrode leads on a sapphire substrate, as shown schematically in Figure 1A-i.²⁷ The electrode leads have metal stacking of Ti/ Ni/Ti/Ni (30 nm/200 nm/50 nm/200 nm) for adhesion/ conduction/diffusion-barrier/silicidation purposes, respectively. When a thin Si chip, 5 mm × 5 mm × 50 μ m, is brought into contact with the metal leads on the sapphire substrate, a



Figure 2. Modeling/characterization/measurement of the electro-neural system on mouse hippocampal neurons. (A) Electrical circuit models and corresponding SEM images of the electrode-cell engulfment. (i) Intracellular electrode configuration. (ii) Extracellular electrode configuration. The electrical circuit model of the electro-neural system starts by the cell culture (yellow) interfacing with the electrode, all the way to the read-out electronics represented by the amplifier block (green). I_{Na} and I_K represents the Na+ and K+ ionic currents, and R_{Na} and R_K are the corresponding ion channel resistances. R_{seal} is the seal resistance at the cell-nanowire interface. R_{EC} represents the resistance of the electrochemical reaction at the electrode tip, and C_{EC} is the double layer capacitance. C_{stray} and C_{amp} are the bare electrode wire and amplifier's input parasitic capacitances, respectively. (B) Spontaneous action potentials recorded on mouse hippocampal neurons. (i) Recordings showing the positive measured signal (~20 mV p-p) (left in blue) and after deconvolution (right in red). (ii) Recordings showing the negative measured signal (~10 mV p-p). Small potential fluctuations were captured in both cases. (C) Effect of external application of glutamate/TTX to the recordingsolution. (i) Baseline recordings showing the normal cell firing activity. (ii) After addition of 196 μ M Glutamate to the recording solution, increased activity is observed. A sample action potential is shown on the right (blue), and its deconvoluted potential is shown below (red). (iii) After the application of 1.5 μ M TTX, the cell activity is blocked. (D) Effect of addition of 13.2 mM KCl to the recording solution (i) Baseline recording showing very quiet cell activity. (ii) Following the application of KCl, increased cell activity was observed with a train of action potentials. Similarly, sample action potential is shown on the left (blue), and its deconvolution (red). Electrophysiology was performed

moderate heat treatment (400 °C) and compressive pressure (around 10 MPa) in a vacuum chamber with forming gas (H₂ 3-3.8% in N₂) flow initiates a diffusion reaction between Ni and Si. The formed NiSi alloy fuses the two substrates together (Figure 1A-ii). The temperature 400 °C was chosen because it led to reacted NiSi leads that formed ohmic-like contacts with the heavily doped Si (Figures S1 and S2). The height of the desired nanowire array can be adjusted by an SF₆ inductively coupled plasma (ICP) and reactive ion etch (RIE) that thins the Si substrate to around 8–10 μ m (Figure 1A-iii). Another electron-beam lithography step patterns Ni etch mask dots aligned exactly at the tips of the Ni leads underneath the Si substrate (Figure 1A-iii). An SF₆/C₄F₈ based ICP/RIE process is used to etch the Si everywhere except regions masked by the Ni dots, leaving vertically standing Si nanowires on NiSi leads (Figure 1A-iv and Figure 1B), which have diameters in the

range of 100-200 nm in this work. To prevent electrochemical reactions anywhere except at the nanowire tips, the entire chip is coated by SiO_2 , which is then selectively etched at the contact pads and nanowire tips (Figure 1A-v, Figure 1C, Figure S4). The scanning electron microscope (SEM) images of the Si nanowire arrays in Figure 1B and C demonstrate a high packing density of 6.25 Million/cm² at a pitch of 4 μ m. We also demonstrated submicrometer pitch at a site-to-site spacing of 750 nm (Figure S3). The array geometry can be tailored for the optimal growth of neuronal networks²⁸ that are interconnected with sealed microfluidic channels that can allow growth of neurites and synaptic connections but prevent cell-body plating inside the channels (Figures S5 and S6). The electrochemical impedance in all of these configurations is relatively uniform (Figure S5 and S6) and validated a capacitively dominant coupling behavior (phase in Figure S7) with neuronal activity.



Figure 3. Recording of a 99 mV action potential and pharmacological experiments for validating subthreshold potentials at 10 DIV. (A) Spontaneous activity measured on channel 44 showing subthreshold oscillations that are illustrated in the insets of a 1 s time window at the beginning of the trace (lower inset) and just at the action potential generation (top inset). (B) Concurrent recording with A from a nearby channel 46 showing some potential oscillations and negative spikes that occur simultaneously with those in A. (C) Recording with a solution containing D-APV (50 μ M), CNQX (10 μ M), and PTX (1 mM) to block NMDA, AMPA, and GABA receptors, respectively, shows a single oscillation prior to the spike on the same channel as in A. (D) Same in C from channel 37 showing no oscillations prior to the large action potential.

Transmission electron microscopy (TEM) and elemental mapping by energy-dispersive X-ray spectroscopy (EDX) of the Si nanowires demonstrate crystalline structures and interfaces and highlight the usefulness of each layer: Si constitutes the main body of the sensor, SiO₂ constitutes the passivation outermost cylinder around the bottom portion of the nanowire (Figure 1D), Ni is used for silicidation bonding and as a current conduction layer (Figure 1E top and bottom, respectively), and Ti is used as a Ni diffusion barrier and adhesion layer (Figure 1F top and bottom, respectively). A zoomed-in TEM image at the bottom of the wire in Figure 1G highlights the interfacial layers crucial for the free-standing and individually electrically addressable nature of the Si nanowire. The high-resolution TEM (HRTEM) images in the lower panels of Figure 1G illustrate the crystalline nature of the interface between NiSi and Si at the bottom of the wire (see also Figure S9). Similar bonding structure, morphology, and interfaces were validated on SiO₂/Si substrates (Figure S10) illustrating the versatility of our bonding scheme.

We next investigated the biological interfaces established between our Si nanowires and neurons and the resulting recorded electrophysiological activity. We packaged our devices on commercial electrophysiology printed circuit boards (Figure S8) and tested their feasibility for electrophysiology and pharmacology using rodent hippocampal primary neurons and human iPSC-derived neurons (Supporting Information sections 3 and 4). For both rodent primary and hiPSC-derived neurons, we find strong interaction between neurons and nanowires characterized by cell outgrowth and engulfment to the vertical Si nanowires (Figure 2A and Figure 4A, primary mouse neurons; Figure S12 uncolored SEM images). The cell– electrode interface is generally established in intracellular and extracellular configurations both of which can provide sufficient coupling between the cell and the electrode to enable high fidelity recordings.

The amplitude and shape of recorded potentials are governed by the electrochemical interface at the surface of the nanowire and the degree of sealing for the cell membrane to the nanowire itself, and by the measurement system.^{29–31} To account for these effects in both intracellular and extracellular configurations (Figure 2A), we developed a circuit model based on experimental measurements for each component of the electrode/neuron interface (see Supporting Information section 6). Results using analytic transfer functions, circuit simulations, and empirical transfer functions for the overall measurement system provided excellent agreement with the measured potentials.

One of the main signatures of our model is the use of current source and different conductance for the Na⁺ and K⁺ channels that is commensurate to the physical origin of the faster depolarization and repolarization in action potentials. These were calibrated to patch-clamp measured inward and outward ionic currents from similar cultured cells (Figure S15a). The longer duration of the patch-clamp action potentials has been previously related to maturity, culture conditions, and exact temperature of the cell culture for both hiPSC³² and rodent cortical neurons,³³ which was also observed in our reference patch-clamp measurements (Figure S15a). Our electrophysiological models using either analog circuit analysis or empirical simulations based on detailed electrochemical characterization, similar to the models of Spira et al.²⁹⁻³¹ are self-consistent models that can accurately reproduce both the time and the amplitude of deconvoluted neuronal signals measured from nanowires.



Figure 4. Recording from hiPSC-derived cortical neurons. (A) Colorized angle-view SEM image showing cell morphology and neurite outgrowth to nearby nanowires. Scale bar is 4 μ m. (B) Measured potentials on channels 6–8 showing positive potentials for wires 7 and 8 inside the cell and negative potential for wire 6 outside the cell. (C) Colorized SEM image of the Pt coated and FIB thinned cross-section of the cell in panel B. The image shows the cell (yellow colored) engulfing the tops of nanowires 7 and 8, and portions of cell membrane around nanowire 6. Scale bar is 2 μ m. Electrophysiology was performed 6 weeks post in vitro culture. (D, E) Higher magnification colorized TEM images collected and stacked to provide a higher resolution close-up to the nanowire-cell interface. The slight bending of the nanowires is likely to have happened during neuron dehydration in preparation for SEM imaging (section 5 of Supporting Information). Scale bars are 200 nm. (F) Recordings on nanowire number 7 illustrating consistent oscillations prior to action potential firing at different recording times.

Pharmacological stimulation and inhibition validated the physiological origin of the measured potentials. After glutamate was added to the recording solution, we observed an increase of the cell spontaneous activity (Figure 2C-ii) with respect to frequency and amplitude when compared to the baseline (Figure 2C-i) measured on the same channel. The bath application of tetrodotoxin (TTX) inhibited the activity on the same channel (Figure 2C-iii). Similarly, for the extracellular configuration, the bath application of KCl led to increased activity (Figure 2D-ii) relative to the baseline recording (Figure 2D-i), which was also eliminated by TTX treatment.

Strikingly, physiological measurements on mouse hippocampal neurons cultured for 10–13 days on our platform displayed small potential fluctuations prior to the positive (Figure 2B-i) and negative (Figure 2B-ii) firing events. The SNR of these prespike potentials is 20-times as evident in the inset of Figure 2B-i, and their shape is clearly different from coupled action potentials from different cells or channels. The largest action potentials that we measured 10 DIV is 99 mV, as shown in Figure 3A, which demonstrates for the first time that nanowires can measure intracellular potentials with similar magnitudes to that of patch-clamp. We generally observed oscillations prior to spikes as highlighted in the insets of Figure 3A. For a simultaneously measured extracellular potential from another channel (Figure 3B) that is 5.65 μ m apart from the intracellular channel, we also observed potential oscillations prior to the extracellular spike. Upon inspecting all other channels, we did not observe action potentials that might be electronically coupled to these two channels. We then embarked on validating that these potential oscillations are subthreshold synaptic potentials. To do so, we pharmacologically blocked both excitatory and inhibitory receptors by adding D-APV to block NMDA receptors, CNQX for blocking AMPA receptors, and Picrotoxin for preventing the binding of the inhibitory neurotransmitter GABA to its receptors (see Supporting Information section 4 (1)). After adding the blockers, we observed insignificant number of small prespike oscillations (Figure 3C) or no oscillations (Figure 3D), which suggested that our system has the sensitivity to detect miniature release of neurotransmitters at a quantal level. Given that our nanowires can sometimes measure intracellular potentials with an SNR of 1700 (Figure 3A), it is not surprising that they can also resolve subthreshold activity that we validate with standard pharmacological experiments here. This can be attributed to the height of our nanowires, which is >6.5 μ m compared to the shorter than 2 μ m nanowires in prior works, providing larger Si surface interaction area with adherent neurons.

The development of high-throughput, subcellular neurotechnologies has the potential for application to drug screening on neurological disease models. We therefore tested the



Figure 5. Recording from hiPSC-derived dopaminergic neurons 6 weeks post in vitro culture. (A) Spontaneous action potentials. (i) Positive potential recordings showing the measured signal (~30 mV p-p) (left in blue) and after deconvolution (right in red). (ii) Negative potential recordings (~6 mV p-p) (left in blue) and after deconvolution (right in red). (B) Potential oscillations recorded prior to action potential firing. (C) Effect of external application of stimulating/inhibiting media. (i) Increase of action potential firing following 13.2 mM KCl bath application. (ii) Increased activity abolished after the application of 1 μ M TTX.

sensitivity of our platform with two clinically relevant subtypes of human iPSC-derived neurons, cortical and dopaminergic, for which we had demonstrated electrical activity on conventional microelectrode array recordings (Figure S11).

Human iPSC-derived cortical neurons cultured on our platform overlapped with multiple nanowires (Figure 4A). Post preparation for SEM on this platform, evidence of neurite outgrowth to nearby nanowires, is also apparent in Figure 4A. Nanowires 7 and 8 displayed positive action potentials, whereas nanowire 6 displayed a negative action potential (Figure 4B). From the SEM image of Figure 4A, we can note the extracellular nature of the interface with nanowire 6. To uncover the nature of the nanowire/neuron interaction for nanowires 7 and 8, we performed a sequential focused ion beam (FIB) cut and thinning of a 300-400 nm slice on the sample in regions of wires marked 6, 7, 8 (Figures S17-S21) post Pt plating. The sample is thin enough to allow electron transmission for TEM characterization without risking significant damage to the cell body during the FIB milling process. Figure 4C shows an SEM image of the FIB slice showing a clear dark contrast of the cell around nanowires 7 and 8. The TEM images in Figures 3D and 4E along the substrate-nanowire-cell regions demonstrated that the nanowires displayed intimate interaction with neurons that is not aided by tension due to neuron adhesion and spreading on the substrate surface or peptide-modification,³⁴⁻³⁶ nor assisted with the highly invasive electroporation.9,10 The continuity of the inclined interface below the SiO₂ passivation layer as seen to the right of the nanowires in Figure 4D and E is suggestive of intracellular penetration of the nanowires into the human cortical neuron cell body, which is commensurate with the positive potentials measured with wires 7 and 8.37 However, it is also possible that the cell fixation and FIB thinning can lead to artifacts in the observed interface. Also in these recordings, we observed the prespike potentials with sharp rise and slow decay times, which are similar in shape to the excitatory postsynaptic potentials (EPSPs) observed by Hai et al. using Au mushroom electrodes.³⁸ It is possible however that the positive potentials are measured in a juxtacellular configuration due to their smaller amplitude than those observed in Figures 2, 3, and

5 since we did not validate their nature by blocking the postsynaptic receptors. We note that out of the two hiPSC cells on top of the nanowire array (Figure S17), we measured positive potentials from one neuron. The individually addressable nanowires can record multiple positive potentials from a single neuron.

Dopaminergic neurons are a clinically relevant cell type for study of neurodegenerative disease and neuropsychiatric disorders. We determined whether hiPSC-derived dopaminergic neurons could also survive and demonstrate physiological function on our nanowire device. Six weeks postplating hiPSCderived dopaminergic neurons on the nanowire platform, we observed distinctive single slope rise potentials compared to the multioscillation behavior exhibited by the mouse hippocampal neurons and hiPSC-derived cortical neurons prior to spontaneous action potential firing for both positive and negative polarities (inset, Figure 5A). On some channels, we observed small potential oscillations with varying frequency and amplitude as shown Figure 5B. We then performed pharmacological studies after 2 weeks postastrocyte coculture on the hiPSC-derived dopaminergic neurons (6 weeks post initial culture). Upon bath application of KCl, the firing rate increased and then all activity stopped after the addition of TTX as shown in Figure 5C. In conclusion, hiPSC-derived neurons not only survived several weeks intimately interfaced with the nanowires, but also we observed extensive electrophysiological activity over time, which led to the possibility of longitudinal electrophysiological experiments on synaptic activity on in vitro human neuronal networks.

The electrophysiological recordings from our cultures did not display trains of large action potentials, but just one or two APs, which is consistent with recordings from immature neurons and network observed on both human and rodent cultures from other groups.^{32,39} Therefore, we performed immunocytochemistry analyses on primary neurons cultured on our platform. The rat cortical neurons fixed at 15 DIV showed that the cells are well adherent to the nanowire arrays, stained positive for Tuj1, a specific neuronal marker, with some limited neurite extension. (Figure S22). The normal formation of a mature neuronal network can be compromised by the height of our nanowires that pin the cells above the planar regions of the platform. This can be improved by creating 3D islands at the bottom of the nanowires that can promote network connectivity on a 3D surface. In the extreme case of shortening the nanowires to heights that are similar to that is reported previously, the intracellular capability of the nanowires might be compromised. Increasing the spacing between the nanowires may also strengthen the network activity of the culture as noted in the recent work of Shmoel et al.⁴⁰ but will compromise their density. Further investigation of the limited neurite extension in our cell culture will lead to improved network synaptic connections and to more robust and consistent measurements of action potentials and PSPs. (Figures 2-4).

It is also important to report the our primary rodent neuron experiments were conducted on 5 devices with successful measurements for several days for both intracellular-like and extracellular-like potentials on 4 out of the 5 devices. The measured intracellular-like potentials varied from 0.1 mV to 99 mV. The hiPSC experiments were conducted on 4 devices with successful measurements for several days for both intracellularlike and extracellular-like potentials on 4 out of the 5 sets, and for one device over several weeks (4 weeks and then 6 weeks after culture). The measured intracellular-like potentials varied from 0.1 mV to 35 mV. From Figure S17, we can observe 2 cells in the 28 μ m × 28 μ m square array of nanowires leading to a cell density of ~255 100 cells/cm². One can also observe from Figure S17 that two out of the 12 nanowires in contact with the cultured cells resulted in intracellular-like measurements. As observed by other groups the variability of the Amplitude and duration of APs can be due to different engulfment level or cleft width between the neuron and the nanowire.⁴⁰ The yield on nanowire internalization to cells can also be improved, for example with peptide modification.³

Measurement of intracellular action potentials using individually addressable Si nanowire probes, from both mouse and human neurons, opens new prospects on mapping neuronal activity in large networks, while the sensitivity to subthreshold postsynaptic potentials from multiple neurons opens new possibilities to study synaptic transmission mechanisms and plasticity, particularly important for investigating neurological diseases. Given the scalability of our arrays, the simultaneous recording of minute changes in cell potentials can uncover details on the synthesis, processing, and execution of neuronal network activity. In vitro, highly parallel drug screening experiments can be performed using the human relevant iPSC cell line and without the need of the laborious nonscalable patch-clamp. In vivo, targeted modulation of individual neural circuits or even single cells within a network becomes possible, and implications for bridging or repairing networks in neurologically affected regions become within reach. Overall, our platform and modified versions thereof have the potential to lead to transformative technologies for both in vivo and in vitro applications.

ASSOCIATED CONTENT

S Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.nano-lett.6b04752.

Detailed information regarding fabrication, packaging, electrochemical impedance spectroscopy, TEM sample preparation, cell culture, pharmacological stimulation and inhibition, SEM imaging, modeling and simulation, and nanowire-cell interface characterization (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: sdayeh@ece.ucsd.edu.

ORCID 💿

Renjie Chen: 0000-0002-3145-6882

Shadi A. Dayeh: 0000-0002-1756-1774

Author Contributions

S.A.D. conceived the integration scheme and platform design, led the project, wrote the manuscript, and received input from all coauthors. R.L., R.C., Y.H., A.T., Y.G.R., X.D., and S.A.D. fabricated the platform with input and equipment training from C.S., A.J., J.N., and D.B.W. R.L. performed the FIB sectioning and TEM characterization with input from D.V.P. and K.L.J.; J.S., S.H.L., and Y.Z. cultured the primary rodent neurons, and R.L., S.H.L., M.L.K., D.P., and S.A.D. performed electrophysiology on these devices. S.H., S.B., and A.G.B. performed the hiPSC culture and MEA analyses, and S.H.L, S.H., M.L.K., and A.K.M. performed electrophysiology on the hiPSC devices. A.T.E. and S.A.D. carried out the electrochemical interface modeling.

Notes

The authors declare no competing financial interest.

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Supplementary Materials

High Density Individually Addressable Nanowire Arrays Record Intracellular Activity from Primary Rodent and Human Stem Cell Derived Neurons

Ren Liu,¹ Renjie Chen,¹ Ahmed E. Elthakeb,¹ Sang Heon Lee,¹ Sandy Hinckley,² Massoud L. Khraiche,¹ John Scott,³ Deborah Pre,² Yoontae Hwang,¹ Atsunori Tanaka,⁴ Yun Goo Ro,¹ Albert K. Matsushita,⁴ Xing Dai,^{1,5} Cesare Soci,⁵ Steven Biesmans,² Anthony James,⁶ John Nogan,⁶ Katherine L. Jungjohann,⁶ Douglas V. Pete,⁶ Denise B. Webb,⁶ Yimin Zou,³ Anne G. Bang,² and Shadi A. Dayeh,^{1,4,7}*

¹Integrated Electronics and Biointerfaces Laboratory

Department of Electrical and Computer Engineering, University of California San Diego, 9500 Gilman Dr., La Jolla CA 92093

²Conrad Prebys Center for Chemical Genomics

Sanford Burnham Prebys Medical Discovery Institute

10901 North Torrey Pines Road, La Jolla CA 92037

³Neurobiology Section, Biological Sciences Division, University of California San Diego, 9500 Gilman Dr., La Jolla CA 92093

⁴Graduate Program of Materials Science and Engineering, University of California San Diego, 9500 Gilman Dr., La Jolla CA 92093

⁵Division of Physics and Applied Physics, Nanyang Technological University

21 Nanyang Link, Singapore 637371

⁶Center for Integrated Nanotechnologies, Sandia National Laboratories, Albuquerque, NM 87185, USA

⁷ Department of NanoEngineering, University of California San Diego, 9500 Gilman Dr., La Jolla CA 92093

*e-mail: sdayeh@ece.ucsd.edu

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1. Fabrication of vertical Si nanowire arrays:

(1) Solid-state wafer bonding between thin Si chip and Ni electrodes on Sapphire wafer

The 50 μ m thick 4" Si substrates are obtained from Virginia Semiconductor Inc. and are carefully handled with plastic tweezers. The samples are diced into 5 mm × 5 mm pieces that are then gently cleaned in piranha solution (H₂SO₄:H₂O₂=3:1). Before the wafer bonding, the cleaned Si samples are dipped in buffered oxide etchant (BOE) to strip the surface oxide layer. On a separate sapphire substrate, photo and e-beam lithography are used to pattern the electrode leads followed by electron beam evaporation of Ti/Ni/Ti/Ni, and the sample is then washed with diluted NH₄OH (1:5 in DI) to remove the native NiOx surface layer. After rinsing with DI wafer and a N₂ gun dry, these two samples are brought together and sandwiched by another two handling sapphire wafers. A binder clip then clamps on these two handling wafers to apply enough compressive pressure (around 10 MPa) during the bonding. The wafer bonding is performed at 400 °C in a vacuum chamber with forming gas (3-3.8% H₂ in N₂) flow for 15 minutes. We used a similar procedure for the reliable integration of planar compound semiconductor layers (InGaAs) to SiO₂/Si substrates.^{1.2}

Figure S1 shows initial bonding tests of Si to sapphire and SiO₂/Si using NiSi formation. The nature of the solid-state reaction permits the realization of isolated NiSi leads underneath the bonded Si substrate (Fig. S1d). The ohmic contact characteristics of the Ni_xSi_y contact with Si was studied on a relatively moderate/high resistivity Si substrate (ρ =0.01 Ω .cm) where the annealing temperature in forming gas was varied from room temperature to 800 °C. As expected, we found that NiSi formed at 400 – 600 °C results in the lowest contact interface resistance for the test structures and the NiSi₂ phase at 800 °C results in higher contact resistances (Fig. S1f).³ Therefore, The low temperature anneal has also the advantage of obtaining low ohmic contact resistances and therefore used in out integration schemes.



Figure S1: a) Cross-section SEM image of a 5 mm \times 5 mm Si substrate bonded to a sapphire substrate. b) Close-up SEM image near one NiSi reacted electrode lead and c) higher magnification SEM showing intrusion of the NiSi into the Si substrate and recession of the Si substrate reducing the air gap between Si and the sapphire substrate. d) NiSi bonding performed on patterned Ni leads on a SiO₂/Si substrate with a zoom-in to the interface at one electrode site (right panel in (d)). e) Test structure for current-voltage characteristics as a function of annealing temperature with Ni being in contact with Si, and d) results showing rectifying behavior at room temperature followed by linear ohmic-like current-voltage characteristics or all annealing temperatures with lowest resistance/highest currents for the 400 – 600 °C annealing reaction temperature.



Figure S2: Transmission electron microscopy of the Ni_xSi_y/Si(111) interface for two annealing temperatures. a) At 400 °C, a NiSi orthorhombic phase is formed. At 800 °C, a cubic NiSi₂ phase is formed that is nearly lattice matched to Si.

(2) RIE/ICP dry etching processes for Si thinning and Si nanowire etching

After the wafer bonding, the 50 µm thick Si chip is thinned down to ~ 10 µm utilizing the RIE/ICP dry etching tool (Oxford Plasmalab 100). SF₆ gas of 80 sccm with RIE/ICP powers of 200 W/1500 W are used for the rapid thinning process (~ 4.2 um/min). During the Si nanowire etching step, following the Ni etching masks, a combination of SF₆ 18 sccm and C₄F₈ 56 sccm gases is introduced for etching nanowires with smooth sidewalls. An RIE/ICP powers of 30 W/1200 W were optimized to control the nanowire etching profile. The Si nanowire etching finishes when all the surrounding Si is removed and an O₂ plasma etch follows to remove polymers that build up during the etching process.



Figure S3: SEM images of etched nanowire arrays on sapphire substrate. a) Angle view SEM image of the overall platform after Si nanowire etching. The width of the platform is 1.5 cm. b) SEM image of Si nanowires etched at a pitch of 10 μ m site-to-site spacing. c) SEM image of Si nanowires etched at a pitch of 4 μ m site-to-site spacing. d) SEM image of a linear array of Si nanowires etched at a pitch of 750nm site-to-site spacing.

(3) SiO₂ layer passivation

The whole device, except the big pads for electrical connections, is coated with 400 nm SiO_2 (Oxford Plasmalab PECVD), resulting ~ 200 nm thick SiO_2 on Si nanowire sidewalls. Then, a thin PMMA layer (~ 200 nm) is applied to the entire device by spin-coating, followed by baking at 170 °C for 10 min. A short O_2 plasma treatment can expose the PMMA at the tip of Si nanowires. After that, a short BOE dip can remove the SiO_2 only at the tip of Si nanowires. Finally, this PMMA layer can be stripped by O_2 plasma cleaning.



Figure S4: Colorized SEM images of Si nanowires etched on top of NiSi leads for a 290 nm tip diameter before (a) and after (b) SiO₂ passivation and back etching, and for a 90 nm tip diameter before (c) and after (d) SiO₂ passivation and back etching. Exposure of the tip can be well controlled mainly with the diluted BOE etch time. Scale bars in all panels are 1 μ m.

(4) Extension to other array geometries and overall passivation with SU8

The integration scheme can be used to tailor the array geometry and to the inclusion of microfluidic/neurite growth channels. It has been demonstrated that hexagonal patterns in SU8 layers provide optimal geometry for neuronal growth to promote axonal growth between neurons plated at the vortices of the hexagonal pattern. ⁴ It has also been demonstrated that thin SU8 channels can trap neuronal growth to the channels, provided that these channels are coated with poly-L-lysine or poly-D-lysine charged molecules. We demonstrated that our integration technology is suitable to define patterned nanowire arrays as shown in Fig. S5 and correspondingly microchannels in 10 µm thick SU8 resist as shown in Fig. S5c,d.



Figure S5: SEM images of Si nanowires arrays arranged in a hexagonal pattern with 7 vortices (a) and zoom-in to a single vortex with 8 nanowires (b). (c) Definition of neuron plating regions with SU8 with optimized processing conditions to seal a microfluidic channel in SU8 that connects the vortices of the hexagon and yet maintains a sealed top to prevent neuron plating in these microfluidic channels. (d) Electrochemical impedance spectroscopy was conducted on all 56 channels which displayed a consistent 1kHz impedance magnitude of ~ 20M Ω as discussed in Fig. S7. Inset of panel (d) is a mechanically displaced SU8 region surrounding a microfluidic channel demonstrating that the sealed channel is indeed well developed and free of SU8 potentially permitting neurite growth between the hexagonal vortices.

The same passivation procedure has also been applied to the square array pattern. Figure S6 shows a square array of Si microwires with 10 μ m pitch that was patterned to allow neural cell plating in the square array region. The electrochemical impedance was also consistent among the 56 channels and with that of the hexagonal patterns (~20M Ω at 1kHz).



Figure S6: Optical microscope image of a patterned SU8 layer on a square array (b) to confine neuron cell plating and electrochemical interactions with the exposed nanowires. The electrochemical impedance of the array nanowires is shown in panel (c).

(5) Electrochemical impedance spectroscopy

Si nanowire arrays with SiO_2 passivation and exposed tips with and without the SU8 patterned layers were subject to 3-terminal electrochemical impedance spectroscopy using a Gamry Instruments potentiostat.⁵ The measurements were conducted in

phosphate buffered saline solution (ThermoFisher Scientific) with a Pt counter electrode (CH129, Chin Instruments) and Ag/AgCl reference electrode (CH111, Chin Instruments). Figure S7 shows a sample measurement for impedance magnitude and phase indicating a mostly capacitive coupling to alternating charge density in the saline solution. The electrodes are therefore referred as capacitive in nature.



Figure S7: Electrochemical impedance spectra for a single Si nanowire. a) Magnitude and (b) phase.

Using a parallel RC model (solution resistance can be safely neglected), we approximated the faradiac component to have a $65M\Omega$ resistance, and the capacitive branch to have 8pF capacitance. This is large given that the exposed tip of the Si nanowire is 1-3µm long. However, given that the SU8 covered leads or non SU8 covered leads resulted in similar impedances, the stray capacitance is less likely to play a role in these measurements and it is likely that the electric field intensification at the nanowire tips contributes to the enhanced capacitance.

(6) Packaging:

After device fabrication, the samples were bonded via conductive epoxy (EpoxySet EO-21M-5) to commercially available Ayanda Biosystems⁶ printed circuit boards that are compatible with conventional neurophysiology stations. Bonding was performed at 80 °C for 3 hours in ambient). Quartz tubes were then bonded with Polydimethylsiloxane (PDMS) to localize the neuron culture chamber. (Fig. S8). Either PDMS or SU8 were then used to tight seal the inner portion of the nanowire array chip and the bottom of the

PCB (Fig. S8) against fluid leakage. Both of these seals were found to be leak-proof over several weeks of neuron culture (up to 8 weeks in our experiments before disassembling the sample for imaging).



Figure S8: a) Picture of packaged nanowire array chip (lower right inset) with details of the different used layers except for the conductive epoxy between the pads on the back of the PCB and the top of the nanowire chip. b) picture of a different device showing a dark region in the center which is the same size of the bonded $5\text{mm} \times 5\text{mm}$ starting Si substrate.

2. Transmission electron microscopy (TEM) samples preparation with focused ion beam (FIB) milling

(1) Sample preparation for FIB milling

To prevent the sample from the damage by ion beam during the FIB milling process, the fabricated device is deposited with $1\mu m SiN_x$ by Trion Orion III chemical vapor deposition (CVD) system. This SiN_x layer also provides better contrast to the passivating SiO_2 dielectric layer on Si nanowire probes for elemental mapping. After that, the sample was coated with a 30nm Pt layer by e-beam evaporation to reduce the charging under electron and ion beams.

(2) FIB milling and in-situ lift-out (INLO)

The TEM sample lamellae was prepared with FIB (FEI Nova 600). The FIB and INLO process utilized here follow conventional procedures, in which a 30keV Ga beam was used for rough milling and reduced voltage (10keV) was used for fine milling.

(3) Transmission electron microscopy (TEM) characterizations:

TEM characterization was carried out using a FEI Tecnai G(2) F30 S-Twin 300kV transmission electron microscope, equipped with Fischione Instruments high angle annular dark field (HAADF) and EDAX ECON energy-dispersion x-ray (EDX) detectors under scanning TEM (STEM) mode. Those experiments were performed at the Center for Integrated Nanotechnologies at Sandia National Laboratories.



Figure S9. TEM characterization and elemental mapping of a single Si nanowire on sapphire substrate. (A) Low magnification TEM image of a single Si nanowire bonding with Ni/Ti/Ni/Ti electrode on sapphire substrate. The sample is coated with additional SiNx and Pt for the purpose of cross-sectioning for TEM studies. Scale bar is 1 μ m. EDX element mapping of Si (B), O (C), Ni (D) and Ti (E), respectively. Scale bars are 1 μ m. (F) Higher magnification TEM image of the Ni/Ti/Ni/Ti electrode, and the interface of Si and NiSi. Scale bar is 200nm. (G) HRTEM image of the interface between Si and Ni at the Si nanowire tip and its Fast Fourier Transform (FFT) pattern in (F). HRTEM images at the center area of Si nanowire (I), and at the interface between Si and NiSi with electron beam axis aligned in the Si [110] (J), and the NiSi [110] Zone axis in (K). Scale bars in G, I, J, K are 2nm.



Figure S10. TEM characterization and elemental mapping of another Si nanowire on sapphire substrate a) HAADF STEM image of a Si nanowire on sapphire substrate. b) Bright-field (BF) image of same nanowire in (a). c-f) elemental mapping showing Si (c), O (d), Ni (e) and Ti (f) composition of the nanowire structure. These characteristics are generally consistent with that in Fig. S9 and other wires characterized on sapphire and SiO₂/Si substrates.

3. Cell Culture:

(1) CA1-CA3 mouse hippocampus neurons

In preparation for plating of mouse hippocampal neurons, plates were incubated over night at room temperature with Poly-D-Lysine (80mg/mL; Millipore) in Phosphatebuffered saline (Cellgro). The following day, plates were washed and incubated with Poly-D-Lysine (40 mg/mL) and Laminin (2.5mg/mL; Invitrogen) in Phosphate-buffered saline for 3 hours at 37C. Culture media was prepared from Neurobasal Medium (Gibco) supplemented with 5% Fetal Bovine Serum (Gibco), 2% B27 supplement (Gibco), 1% Glutamax (Gibco), and 1% Penicillin/Streptomycin (Cellgro).

Hippocampi were isolated from E18 mouse embryos from pregnant CD1-IGS mice (Charles River Laboratories). Cells were dissociated by incubation at 37C in a 0.25% Trypsin/ 2.21mM EDTA solution (Corning) and triturated by pipetting. After pelleting heavy debris, the cell solution was diluted to a density of 1 x 10^6 cells/mL and 200µL of the resulting solution was plated directly onto the coated nanowire array. Additional culture media was added to bring the total volume to 1.5-2mL. Cultures were maintained at 37°C in an atmosphere containing 5% CO₂. Half of the culture media was exchanged

every 3 days. Cytosine arabinoside (AraC; working concentration 4μ M) was added from the 6th day of culture to suppress the proliferation of glial cells.

(2) Human induced pluripotent stem cell (hiPSC) derived neurons

All human stem cell culture was performed under approval from the Stem Cell Research Oversight (SCRO) panel at Sanford Burnham Prebys Medical Discovery Institute. Undifferentiated human pluripotent stem cells (generous gift from Dr. April Pyle, UCLA), were cultured on irradiated mouse embryonic fibroblasts in hESC media (DMEM/F12, 20% Knockout Serum Replacement, 1X non-essential amino acids, 110 µM beta-mercaptoethanol, penicillin-streptomycin, and 10ng/ml bFGF). Cortical neurons were differentiated from hiPSC with an established protocol as previously described^{7,8}. HiPSC-derived cortical neurons were treated with 5 µM cytosine arabinoside and passaged onto the nanowire device or multielectrode array plate (Axion Biosytems). HiPSC-derived dopaminergic neurons (iDopa neurons, Cellular Dynamics International) were thawed according to manufacturer's instructions and plated directly onto the nanowire device. Human astrocytes were added to the hiPSC-derived dopaminergic neurons one week prior to recording (ScienCell Research Laboratories). The nanowires or multielectrode arrays (MEA) were pre-coated with 0.1% polyethylenimine (PEI) and hiPSC-derived cortical or dopaminergic neurons plated in Neurobasal A, 10% Knockout Serum Replacement and 10 µg/ml Laminin. Neuronal cultures were maintained in Brainphys media (Stemcell Technologies) optimized for electrophysiological recordings⁹ in a humidified 37°C incubator with 5% CO₂. 60% media was exchanged every other day.

(3) Primary rat cortical neurons

The nanowire array devices have been coated with 0.1% PEI for 1h at room temperature, followed by several washes in PBS and left overnight in the hood for drying. The following day 1:50 laminin solution in PBS have been added for 1h at 37C, and then removed right before the plating. Commercially available primary rat cortex neurons (Gibco) were plated on the nanowire array at a density of 200000 cells each device following manufacturer's instruction, and they were kept in Neurobasal medium (Gibco)

with 1:100 Glutamax-I (Gibco), 1:50 B27 (Gibco) and 1:100 Penicillin/Streptomicyn (Mirus). Half of the medium was changed every three days for the duration of the experiment.

4. Pharmacological stimulation and inhibition

(1) Neurophysiology recording with nanowires

Measurements were conducted on a Tucker Davis Technologies (TDT) RZ2 neurophysiology system equipped with a PZ5-64 channel neuro-digitizing preamplifier, RS4 data streamer, and an MZ60 multielectrode array headstage and amplifier. Mouse hippocampal neurons were recorded in aCSF (NaCl 119mM, KCl 2.5 mM, NaH₂PO₄ 1mM, NaHCO₃ 26.2mM, Glucose 11mM, MgCl₂(7H₂O) 1.3mM, CaCl₂(2H₂O) 2.5mM) and for some recording sessions, the solution was bubbled with 95%O₂ + 5%CO₂ gas for at least 30 minutes prior to recording and also during the recording session. HiPSC-derived neurons were maintained in Brainphys media as the recording solution. 2-3 minutes of baseline measurement was conducted to observe spontaneous neural activity. Then 13mM KCl was added to the device chamber and recorded for 2-3 minutes to observe pharmacologically stimulated neural activity. To prove the physiological potentiation of the electrophysiological activity of the cells, we added 197 μ M of Glutamate to the recording solution/medium. Lastly, 1 μ M tetrodotoxin (TTX) was added to the solution to inhibit neural activity.

For some experiments, after the first 2-3 minutes of baseline recording, the glutamatergic and the GABAergic receptors have been blocked by adding 50 μ M D-APV (Abcam), 10 μ M CNQX (Sigma) and 1 mM Picrotoxin (Tocris) in the medium. The cells have then been recorded in the presence of the blockers. Afterwards the cells have been washed by changing the medium 5 times, 5 minutes each wash, and then recorded again postwashout.

After recording, neurons grown on nanowires devices were processed for immunocytochemistry and/or SEM and/or TEM imaging.

(2) Neurophysiology recording with reference multielectrode array

hiPSC-derived cortical neurons were plated on MEA plates composed of 48-wells with 16 electrodes per well (Axion Biosystems). Recordings were acquired with the Maestro and Axion Integrated Studio. A butterworth band-pass (10-2500Hz) filter and adaptive threshold spike detector set to 5.5X standard deviations were applied to the raw data. Raster plots of neuronal spiking activity were generated using Axion Neural Metrics Tool. Neurons were grown and recorded in Brainphys media. Spontaneous activity was measured as baseline, followed by pharmacological treatment with 2mM KCl and then 1 μ M TTX (Fig. S11).



Figure S11: Reference measurements using commercial Axion planar electrodes/neurophysiology Similar patterns system. of firing baseline, in KCl stimulation, and TTX inhibition to those measured using the Si nanowire array platform were observed.

5. SEM imaging on neurophysiology platform

To prepare the nanowire platform for SEM imaging, the growth media was rinsed with PBS for three times. Subsequently, the samples had undergone a cell fixation protocol, in which a solution containing 2.5 % glutaraldehyde in 0.1 M cacodylate buffer at pH = 7.4 was added. The sample was let sit for 1 hour at room temperature and was the washed

three times in PBS with leaving the sample in PBS solution for 5 min after each rinse. The samples were then rinsed off buffer salts with three times wash in distilled water, 5 min for each rinse. The samples were then subjected to a dehydration procedure in which they were serially dehydrated in 30, 50, 70, and 90% (10 min each) and three times with 100% ethanol. Following the dehydration procedure, the samples were dried in critical point dry for ~10-15 minutes and were then sputter coated in an Emitech K575X coater (5-7 sec at 85mA) with <10nm Ir.



Figure S12: Same as Fig. 2 of main text with SEM images in A.i and A.ii presented as imaged in the electron microscope.

6. Modeling of the Electro-Neural Interface

Following the electro-physiological measurements, further analysis is required to retrieve the actual amplitude and time-scale of the recorded signals by removing signal degradation effects of the measurement device and system, particularly when the sensors themselves are capacitive. This procedure entails a) electrical modeling of the overall system in terms of electrical components - mainly resistors (R) and capacitors (C) starting with the neuron cell membrane, the electrolyte/electrode interface, the electrode's parasitic components, and reaching the read-out electronics (Fig. S13); b) signal processing by applying a deconvolution method to reverse the undesired filtration effects quantified by equivalent transfer function (TF) that is derived in the first step.

Starting with the neuron cell, the basic functional properties for the neuron's membrane during firing an AP, the signal of interest, can be modeled by resistors (R_{Na} and R_K representing the Na+ and K+ ion channels respectively), current sources (I_{Na} and I_K representing the Na+ and K+ ionic currents flow), and capacitor (C_m representing the lipid bilayer of the membrane), where ($C_m \sim 1 \,\mu\text{F/cm}^2$).¹⁰

Upon culturing the neuron cells on the NW electrode's array, the interface with the recording electrode is formed. The quality of the interface (and essentially its electrical properties) is primarily determined by the specific cell-electrode engulfment configuration, which can be quantified by a seal resistance to ground (R_{seal}). Typically, R_{seal} ranges between 100 M Ω to a few G Ω .

The charge transfer across the interface (between the electrolyte and the electrode) is described by the electrochemical process at the tip of the electrode which boils down to a parallel RC branch.

 C_{EC} represents the double layer capacitance, and R_{EC} represents the resistance for the Faradiac charge transfer process or the electrochemical redox reaction at the interface. R_{EC} and C_{EC} were determined from a three-terminal electrochemical impedance spectroscopy measurement of the NW electrode immersed in a saline solution described above (Fig. S7). The values for R_{EC} and C_{EC} were found to be 65 M Ω and 8 pF. A stray capacitance (C_{stray}) representing the equivalent parasitic capacitance of the electrode is estimated to be ~ 80 pF. In addition, the input capacitance of the buffer amplifier ($C_{amp} = 5.5 pF$) is added.



Figure S13. Equivalent Electrical Circuit Model for the Neural Recordings System. The circuit schematic starts with the neuron cell membrane showing the ionic currents $(I_{Na} \& I_K)$, the Sodium and Potassium ion channels represented by R_{Na} and R_K respectively, and the membrane capacitance C_m . The seal resistance (R_{seal}) defines the quality of the interface between the neuron cell membrane and NW electrode and therefore the amount of leakage/signal loss. The interfacial impedance of the electrochemical reaction between the electrolyte and the electrode is represented by the parallel RC branch ($R_{EC} \& C_{EC}$). The schematic includes also the parasitic capacitance which consists of the stray capacitance of the electrode (C_{stray}), in addition to the input capacitance of the buffer op-amp (C_{amp}).

First, examining the model qualitatively enables us to build an intuitive understanding for the overall behavior of the network and the main sources of loss in the electrical coupling. Looking into the model, it can be noticed that the electrical coupling efficiency is impacted in two ways: (a) having a seal resistance creates a current divider in the electrical model, which leads to a significant loss in the measured signal especially in the situations where the cell engulfs the electrode loosely leading to a small R_{seal} values, (b) the parasitic components of the network form an equivalent low pass filter (LPF) with specific time constant (cut-off frequency) that leads to signal spreading. As the values of such parasitic components increase, the effective time constant gets larger and consequently the TF encounters a faster decay associated with higher attenuation (representing the 2^{nd} source of loss in the coupling efficiency) as a result of decreasing the cut-off frequency leading to further spreading in the time scale.

Moving to quantitative analysis, the model is first verified by means of electrical simulations carried out using CADENCE circuit simulator. Typical patch-clamp measured Na+ and K+ currents profiles (sampled from similar cells) were used as input sources to the equivalent electrical circuit model (Fig. S13).¹¹ The quantitative analysis is carried out by providing the specific values of each electrical component in the model based on the respective measurement/calculation. Upon adjusting the values for all the components in the electrical network, the model's accuracy was verified by comparing its output waveform against the measured signals where an excellent matching in both amplitude and time scale were achieved, as shown in Fig. S14, d, i. A slight discrepancy was noticed though for some of the recordings which will be discussed in detail at the end of this section.



Figure S14. CAD Simulation of the Electrical Model on hiPSC-derived cortical neurons. (a) Typical patch-clamp measured Na⁺ and K⁺ currents samples [ref. 11] were used as input sources to examine the equivalent electrical circuit model in CAD simulation tool (CADENCE), this panel shows the net current assuming inward Na⁺ current and outward K⁺ current. (b) Circuit schematic of the electrical model exported from CADENCE simulation tool (using current source to voltage source transformation) for both the intracellular and extracellular interfaces. (c) Sample intracellular transfer function describing the effect of the model on the measured signals. (d) Comparison between actual recorded APs and the equivalent electrical circuit model output based on typical input ionic currents, i. Extracellular, ii. Intracellular. The measurements were performed 6 weeks post in-vitro culture.

An analytical transfer function is then derived describing the impact of the model on the measured signals. For the intracellular case, using the equivalent model shown in Fig. S.13, it can be proved that:

$$TF = \frac{V_o}{V_{m, intra}} = H(S) = H_1 + H_2,$$
$$H_1 = \frac{Z_{Cp}}{Z_{Cp} + Z_{EC}},$$
$$H_2 = \frac{Z_{Cp}}{Z_{Cp} + Z_{EC}} \left[\frac{-1}{(1/Z_m \left(\frac{1}{R_{seal}} + \frac{1}{Z_{Cp} + Z_{EC}} \right) + 1)} \right],$$

where Z_{Cp} is the impedance of the total parasitic capacitance, Z_{EC} the impedance of the electrochemical branch, and Z_m is the equivalent impedance of the cell membrane. A deconvolution algorithm (Fig. S15) is used to reverse such effects quantified by a transfer function that leads to reliable retrieval of the original signals (Fig. S15). The deconvolution process can be carried out in the frequency domain (FD) or time domain (TD). In FD, the algorithm starts by converting the measured signal into FD by performing Fourier Transform (FFT), then using the derived TF, the deconvoluted signal waveform is estimated. Finally inverse Fourier Transform (iFFT) is performed to convert the deconvoluted signal into TD.

To account for the suspect variations within the NW electrode array, a transfer function is derived for each electrode capturing its specific interface and the associated parasitic components, and then the deconvolution process uses the corresponding TF for each channel recordings.

To investigate the multi-phase components of the measured action potentials, we remind that for a typical AP, the profile consists of two phases, the first is positive (crossing the equilibrium level upward) as a result of a depolarization due to the inward Na+ current flow, and then a repolarization starts due the outward K+ current flow leading to the hyperpolarization forming the second (negative) phase (crossing the equilibrium level downward). After that the potential gradually approaches the resting potential coming from the negative phase driving the cell back to equilibrium. It was noticed in our measurements that some recordings on some channels show a third phase in the measured signal profile where the potential crosses the equilibrium level one more time upward forming a third (second positive) phase before resting. Using the above shown passive electrical model, such effect was not captured in the output response leaving some discrepancy with respect to those recordings with high peaking effect, Fig. S14, d, ii.

Careful characterization for the overall recording system including the NW electrodes was carried out. As shown in Fig. S15, a train of square pulses is applied into a saline solution where the NW electrodes are immersed (without the presence of neuron cells) and interfaced with the rest of the measurement system. The system response to the characterization signal is recorded in the same way. Based on the input signal and output response, a transfer function is extracted. An empirical form can be shown as follows:

$$H(S) = \frac{86.02S + 1103}{S^2 + 105.6S + 1488}$$

The absolute of H(S) in FD along with the impulse response are shown in Fig. S15. This technique provides a second experimental approach in deducing the system TF that can be used for signal retrieval similarly through the deconvolution algorithm as illustrated in Fig.S16. Looking at the absolute of the experimentally estimated TF with frequency, a clear peaking effect can be noticed.

A potential implication of having such peaking effect is introducing extra oscillations in the response before going to equilibrium, which might justify having a third phase in some of the recorded signals before resting to equilibrium.



Figure S15. Characterization and Signal Processing. (a) Electrical response characterization setup showing the pulse generator is applied to the saline solution where the NW electrode array is immersed and attached to the measurement system from the other side emulating real recording session, (b) Recorded output of the characterization setup for multiple electrodes. (c) Block diagram illustrating the relation between the input characterization signal, the output signal and the desired TF.



Figure S16. Extracted Transfer Function and Deconvolution Algorithm. (a) The absolute of the extracted TF with frequency showing peaking effect, the right panel is the impulse response showing the time domain representation. (b) Block diagram illustrating the general deconvolution algorithm in FD, (c) Sample measured intracellular signal from mouse hippocampal neuron 8 days post in-vitro culture showing extra oscillation prior to resting and deconvoluted result using the extracted TF shown below where we can notice the overshoot effect is disappeared.

7. TEM characterization on electrophysiologically measured devices

The transmission electron microscopy on electrophysiologically measured devices and their preparation and imaging procedures are similar to those presented in section 2. We show below sequences of images in the preparation of the sample characterized and presented in Figure 4 and its TEM images from the back-side.



Figure S17. Top-view SEM image of the overall device with two hiPSC-derived cortical neurons measured 2 weeks post in-vitro culture (Figure 3) with measured signals that have signals above noise level overlaid on the respective nanowires. We did not observe simultaneous spiking in the two cells for these two neurons, and therefore the measured potentials are displayed at two different times. The scale bar in $4\mu m$.



Figure S18. Sequential SEM imaging before (top row) and during (rest of SEM images) the FIB thinning of wires 6, 7 & 8 marked with a rectangular box in the top SEM images.



Figure S19. Top view SEM image of the FIB thinned sample prepared for TEM characterization.





Figure S20. TEM characterization from the back-side of the sample whose data was presented in Fig. 4 of the main text (Fig. S12, Fig. S13 above) with zoom-in details for each wire. The imaging from the back also confirms the intimate cell-nanowire interface.



Figure S21. Uncolored version of the electron microscope images of Fig. 3 of main text of the manuscript.

8. Immunocytochemistry

After recording, the cells have been fixed in paraformaldehyde 4% for 12 minutes at room temperature. After washing the PFA three times with PBS, the cells have been blocked in 5% Donkey Serum and permeabilzed in 0.1% Triton-X. They have been stained with mouse Neuronal Class III β -Tubulin (Tuj1) (*Covance*, 1:250 in blocking buffer) overnight at 4C. The following day the primary antibody was washed off several times in PBS, and the secondary antibody (mouse Alexa Fluor 488) was added 1:500 in blocking buffer for 2 hs at room temperature. The secondary antibody was then washed several times in PBS and the DAPI (Thermofisher, 1:2000 in PBS) was added for 30 minutes at room temperature. The samples have been imaged with an Inverted IX81 Olympus Compound Fluorescence Microscope.



Figure S22. Images from immunocytochemistry analyses of rat cortical neurons fixed at DIV 15. The images have been taken with an Inverted IX81 Olympus Compound Fluorescence Microscope and a 60x objective. (A) Differential interference contrast (DIC) image. (B) DAPI nuclear staining. (C) The cells have been stained for Tuj1, a marker specific for neurons. (D) Merged image.

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