Bioinspired neuron-like electronics

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As an important application of functional biomaterials, neural probes have contributed substantially to studying the brain. Bioinspired and biomimetic strategies have begun to be applied to the development of neural probes, although these and previous generations of probes have had structural and mechanical dissimilarities from their neuron targets that lead to neuronal loss, neuroinflammatory responses and measurement instabilities. Here, we present a bioinspired design for neuron probes—neuron-like electronics (NeuE)—where the key building blocks mimic the subcellular structural features and mechanical properties of neurons. Full three-dimensional mapping of implanted NeuE-brain interfaces highlights the structural indistinguishability and intimate interpenetration of NeuE and neurons. Time-dependent histology and electrophysiology studies further reveal a structurally and functionally stable interface with the neuronal and glial networks shortly following implantation, thus opening opportunities for next-generation brain-machine interfaces. Finally, the NeuE subcellular structural features are shown to facilitate migration of endogenous neural progenitor cells, thus holding promise as an electrically active platform for transplantation-free regenerative medicine.

The design of materials using concepts inspired by and/or mimicking biology is an attractive strategy for the development of innovative materials and has led to exciting advances across a variety of fields, including controlling crystallization kinetics, developing mechanically tough materials and engineering the design and functionality of biomaterials1,2. Biomaterials have also been used in the development of multifunctional neural probes that will help to understand the brain3,4. Recently, the design of implanted neural probes has begun to benefit from the advantages and opportunities afforded by bioinspired and biomimetic design strategies5. These strategies include designing a stimuli-responsive material with dynamic stiffness6 for easy insertion of probes, and utilizing vein compression to reduce tissue trauma6. In view of these successes in implementing bioinspired concepts in neural interfacing, it is noteworthy that neural probes that adapt the structural or mechanical features of cells have not been explored.

Evidence suggests that structural and mechanical differences between neural probes and neuron targets in the brain can lead to disruption of the native tissue9,10,11 that negatively impacts the capability to stably interrogate and modulate natural physiological activity over time9,10,11. Research aimed at reducing these disparities has focused on improving mechanical properties by optimizing the device geometry2,12,13,14,15 and using more flexible materials2,3, including mesh probes that achieve tissue-like flexibility2,3. Nevertheless, in terms of structure and mechanics, existing probes remain essentially ‘foreign’ to the primary neuron building blocks of the brain, presenting a dichotomy with the successful concepts of bioinspired design.

Design and characteristics of neuron-like electronics

As a step towards biomimetic electronics that address the baseline distinctions between implanted probes and the fundamental neuron component of the brain, we have focused on a probe unit building block that is structurally and mechanically similar to a neuron at the subcellular level (Fig. 1a), although not yet with the functional complexity. In our biomimetic design the sizes of the metal recording electrode and interconnect, which constitute the probe building block, match those of the soma and neurite of a typical pyramidal neuron. Moreover, the interconnect and axon have similar flexibility (details are provided in the following) and the thin polymer insulation is analogous to the myelin sheath; both assist in the propagation of electrical signals from the electrode and soma, respectively. Finally, the neuron-like building blocks are organized to construct an open three-dimensional (3D) electronic network—neuron-like electronics (NeuE)—with structure and topology similar to neural networks (Fig. 1a, inset).

The NeuE was fabricated using photolithography2, where the neurite-like interconnects consist of polymer/metal/polymer structures with polymer and metal widths of 4 and 2 μm or 1 and 0.6 μm, respectively, with a total thickness of ~0.9 μm for the two designs studied (Supplementary Table 1 and Methods). The soma-like platinum recording electrodes were supported on the lower polymer layer (Supplementary Fig. 1). Images of a pyramidal neuron and the two different building blocks used for the NeuE (Fig. 1b, Supplementary Fig. 2a,b and Methods) highlight the biomimetic structural characteristics, including the similarity in sizes of the smaller unit interconnect with dendritic branches. The fabrication of NeuE is detailed in the Methods and key probe layers are shown in Supplementary Fig. 1.

A key physical consequence of the neuron-like dimensions of the NeuE building blocks is that the mechanical stiffness is reduced 5–20 times compared to the most flexible state-of-the-art reported probes16,17,19,20 due to the reduction in feature widths to match the diameter of neurites (Fig. 1c and Supplementary Note 1). The calculated bending stiffness of the NeuE neurite-like interconnect is comparable to the axon of a neuron (Fig. 1c and Supplementary Note 1)2,16,17, thus matching the flexibility of a subcellular component for the first time. Also, the estimated percentage of occupied volume for the implanted NeuE probes, 0.07–0.3% (Supplementary Table 1 and Supplementary Note 2) for the designs in Fig. 1b, is distinct from conventional solid probes16,17 that exclude all cells from their occupied volume, and is up to 20 times lower than recent macroporous}

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Fig. 1 | Design and characterization of NeuE, and 3D mapping of its neural interface. **a**, Schematics showing the structural similarity between NeuE and neurons from the subcellular level to the network level (inset). Neurons, green; electrodes and interconnects, yellow; polymer layers, red. **b**, Fluorescence microscope image of a neuron (I) and false-coloured scanning electron microscope (SEM) images of two NeuE designs (II and III). Raw SEM images are shown in Supplementary Fig. 2. Scale bars, 10 μm. **c**, Bending stiffness of axons, NeuE and examples of previously reported state-of-the-art mesh17 (circle), fibre19,20 (triangle) and thread22 probes (squares). **d**, 3D reconstructed interface between neurons (green) and NeuE (red) at 6 weeks post-implantation. Scale bar, 200 μm. 3D mapping was repeated on N = 3 independent samples. Additional fluorescence images and quantitative analyses are shown in Supplementary Figs. 6 and 7. **e**, High-resolution images of the volumes highlighted by magenta (I), yellow (II) and cyan (III) dashed boxes in **d**. Electrodes are indicated by white dashed circles. Scale bars, 50 μm. **f**, Close-up images of the white dashed box in **d**. I and II correspond to standard fluorescence and depth-coded images, respectively. III and IV are close-up views indicated by the white and grey boxes in I and II, respectively, highlighting the junction between neurites and the NeuE neurite-like interconnect (white dashed circles). Colour codes for depth are shown in the frame in II and the colour bars to the right. Scale bars, 100 μm (I and II), 20 μm (III and IV). **g**, Close-up 3D neural interface of the smaller NeuE (b, III) in additional independent samples near cornu ammonis 1 (CA1) (I) and dentate gyrus (DG) (II) at 2 weeks post-injection. White asterisks indicate dendritic branches. Scale bars, 20 μm.
Similar structure and 3D interpenetration with neurons

We asked how these unique features of NeuE probes affect the interface with neurons within the brain by full 3D mapping of the probe and surrounding brain tissue with subcellular resolution (Supplementary Fig. 3 and Methods) at different times post-implantation. We covalently bound a rhodamine derivative within the bulk of the polymer layers of the NeuE probes to provide stable conjugation and fluorescence under physiological conditions (Supplementary Fig. 4 and Methods). Precise targeted delivery of NeuE probes into live mice was performed using a stereotaxic injection method as described previously (see Methods)\(^{12}\). The NeuE probes were implanted into the hippocampus (HIP) through the cortex (CTX) of transgenic mouse lines expressing either yellow fluorescent proteins (YFPs) in neurons (Thy1-YFP-H)\(^{28}\) or green fluorescent proteins (GFPs) in astrocytes (GFAP-GFP)\(^{29}\). A representative 3D reconstructed image of the NeuE and neurons at 6 weeks post-injection (Fig. 1d and Supplementary Video 1) highlights the similar sizes of the neurite-like interconnects and neuron neurites, the flexibility of these biomimetic ‘neurites’ and interpenetration of the NeuE and neurons from CTX through the CA3 region of the HIP. These and other key features are even more evident in higher-resolution images of the CTX (Fig. 1e.I), CA1 (Fig. 1e.II) and CA3 (Fig. 1e.III), which show the structural similarity between recording electrodes and neuron soma, as well as the interpenetrating interconnects and neurites. The capability to spatially resolve electrodes (white dashed circles in Fig. 1e) relative to surrounding neurons in situ with micrometre resolution allows for correlating structural and functional mapping, as will be discussed later, and has not been possible previously for implanted multi-electrode neural probes.

The structural similarity and interpenetration of the NeuE probe with neurons were further examined by colour-encoding their positions as a function of depth within 3D images. Comparison of zoomed-in views of CA1 with standard fluorophore (Fig. 1f.I) and depth-coding (Fig. 1f.II and Supplementary Video 2) indicates that NeuE integrates in neural tissue without noticeable disturbance, and, indeed, it is difficult to distinguish the interwoven NeuE and neuron structures. Examination of neurite-like interconnects and neuron neurites (Fig. 1f.III,IV and Supplementary Video 3) highlights this latter point, which emphasizes the structural biomimetic nature of the NeuE, and strikingly shows that neuron neurites and NeuE neurite-like interconnects make close contact that might in future be developed into artificial neuro-electronics junctions or synapses. In addition, 3D images (Fig. 1g) of the smaller NeuE design (Fig. 1b,III) at 2 weeks post-implantation show similar integration, with the smaller neurite-like interconnects being comparable to dendritic branches (white asterisks), and show that the overall NeuE probes exhibit greater flexibility within the tissue, consistent with its smaller bending stiffness.

Time-dependent brain interface

We also asked how the implanted NeuE affects the distribution of key cell types across the HIP and CTX as a function of time post-implantation in Thy1-YFP-H and GFAP-GFP transgenic mice, which have been previously validated for assessing tissue integration and foreign body response on the basis of in vivo and in vitro imaging studies\(^{22,28,29}\). Histology imaging and quantitative analyses have been replicated on multiple \(N=3\) independent samples containing NeuE probes at 2 days, 2 weeks, 6 weeks and 3 months post-implantation for both transgenic mouse lines, and show similar results, as detailed in the following (Fig. 2 and Supplementary Figs. 5–7). Representative 3D images of NeuE/neuron (Fig. 2a and Supplementary Fig. 5a) and NeuE/astrocyte (Fig. 2b and Supplementary Fig. 5b) interfaces show a relatively uniform cell distribution without obvious depletion of neurons or enhancements of astrocytes near the NeuE from the earliest time of analysis onward.

We quantified the time-dependent post-implantation response by analysing the cell distributions in volumes containing NeuE and the dentate gyrus (DG), CA1 or the CTX (Fig. 2c and Supplementary Note 4). Representative tissue volumes (Supplementary Fig. 8a) encompass the NeuE probe and most of the cells in each of these three brain regions. Although some displacement of existing tissue occurs during insertion of the glass capillary, which is withdrawn from the brain tissue during injection of the NeuE, normalized neuron fluorescence intensity (Fig. 2c) demonstrated there is substantial neuronal density in the interior of the NeuE probe as early as 2 days. Moreover, the neurons exhibit a fully endogenous distribution in the DG, CA1 and CTX regions at times extending from 2 weeks. Analyses of astrocyte labelled samples (Fig. 2d) further show that the astrocyte density is close to baseline at 2 and 14 days, and is uniform at endogenous levels at longer times. These results for neurons and astrocytes are quantitatively similar to control samples of the same brain regions in the absence of implantation (Supplementary Fig. 9 and Supplementary Note 4). We repeated the time-dependent histology studies for two distinct sets of Thy1-YFP-H and GFAP-GFP mice at 2 days and 2, 6 and 12 weeks (16 mice in total; Supplementary Table 2). Representative fluorescence images (Supplementary Fig. 6) and quantitative analyses (Supplementary Fig. 7) demonstrate the reproducibility of these results. Comparison of the normalized fluorescence intensity from tissue volumes (Supplementary Fig. 8a) versus thin horizontal slices (Supplementary Fig. 8b) relative to the NeuE probes for both neurons and astrocytes also show similar overall results, although there are substantially smaller fluctuations in the fluorescence intensities analysed on the 3D volume samples (Supplementary Fig. 8c,d).

In addition, the samples shown in Fig. 2b and Supplementary Fig. 5b were subject to tissue clearing\(^{30}\) followed by microglia immunostaining (see Methods). Fluorescence images (Supplementary Fig. 5c) and quantitative fluorescence intensity analyses (Supplementary Fig. 5d) show a uniform distribution of microglia around NeuE probes through the CTX, CA1 and DG over this time period, consistent with the absence of an immune response inferred from analyses of neuron and astrocyte distributions. Additionally, sham injection control experiments and subsequent immunostaining (Supplementary Fig. 10 and Methods) show a minimal immune response, as revealed by the close to endogenous levels of astrocytes and microglia at the site of injection. These data contrast the typical depletion of neurons and proliferation of astrocytes and microglia close to the external boundary of solid probes\(^{6,11}\), as well as the decrease in neuron density within the interior and increase in astrocyte density at the surface boundary of conventional macroporous mesh probes even after 2 weeks post-implantation\(^{16}\). Hence, the full 3D analyses provide a quantifiable measure of the substantially lower invasiveness of the NeuE probes. We suggest that the bioinspired design, which mimics the size features and mechanical properties of neurons as well as occupying less than 0.3% volume once implanted (Supplementary Table 1), is crucial to achieving the observed results. Specifically, these features may allow for the migration of cells and the diffusion and dispersion of pro-inflammatory signalling proteins\(^{32}\) that ultimately leads to this attractive behaviour of not activating astrocytes or microglia.

Stable single-unit recording following implantation

We asked whether it would be possible to follow the activity of the same neurons from the time of implantation onwards and, moreover, to correlate the measured activity with the subcellular resolution...
Fig. 2 | Time-dependent 3D histology studies of NeuE-brain interfaces. a, 3D interfaces between NeuE (red) and neurons (green) at 2 days (I), 2 weeks (II) and 3 months (III) post-implantation. Scale bars, 100 µm. b, 3D interfaces between NeuE (red) and astrocytes (cyan) at 2 days (I), 2 weeks (II) and 3 months (III) post-implantation. Scale bars, 100 µm. c, d, Normalized fluorescence intensity of neurons (c) and astrocytes (d) as a function of distance from the 3D NeuE boundary at CTX (orange), hippocampal CA1 (magenta) and DG (blue) at 2 days (I), 2 weeks (II), 6 weeks (III) and 3 months (IV) post-implantation. The pink-shaded regions indicate tissue volumes within the interior of the NeuE. The relative signal was obtained by normalizing the fluorescence intensity with the baseline value defined as the fluorescence intensity averaged over a range of 480–500 µm away (grey dashed horizontal lines; Supplementary Note 4). The tissue volumes for analysis depend on the specific structures of the distinct brain regions, as shown in Supplementary Fig. 5a,b. All error bars reflect ±1 s.e.m. There is substantial neuronal density, 91 ± 12% (mean ± s.d.) of baseline, in the interior of the NeuE probe boundary as early as 2 days, and this increases to 100 ± 7% of baseline at times extending from 2 weeks when the neurons exhibit a fully endogenous distribution in the DG, CA1 and CTX regions. The astrocyte density is 107 ± 8% of baseline at 2 and 14 days, and is uniform at the endogenous level, 102 ± 3%, at longer times. Time-dependent 3D histology studies have been repeated on N = 3 independent samples; additional fluorescence images and quantitative analyses are shown in Supplementary Figs. 6 and 7.
topology of the imaged NeuE and neurons in 3D. NeuE probes were implanted with 16 addressable electrodes distributed across the HIP and CTX, and the probe–brain was imaged at the 3 month recording endpoint to spatially resolve the barcoded electrodes with respect to the surrounding neurons (Supplementary Fig. 11 and Methods). Multiplexed recordings (Fig. 3a and Supplementary Fig. 12) exhibit stable single-unit spikes across the HIP through the CTX regions from the time of initial implantation (day 0) through to the endpoint of the measurements (day 90), where the position of the NeuE probe was fixed following initial implantation. This contrasts with the position adjustments often used to reacquire active neuron signals between recording sessions for conventional probes33,34. Overall analyses of the single-unit spike signal-to-noise ratios (SNRs; Supplementary Fig. 13) show nearly constant values from the time of implantation onward. In addition, the electrode interfacial impedances exhibited relatively constant values over time, and the interchannel impedances show minimal crosstalk between channels (Supplementary Fig. 14 and Methods).

Given this global single-unit recording stability, we asked whether it was possible to illuminate the single neuron activity as a function of time by tracking isolated neurons (Supplementary Note 5). A comparison of sorted spikes from each channel for days 0, 7, 14 and 90 (Fig. 3b and Supplementary Fig. 15) highlights several points. First, the isolated neurons, which are highlighted by different colour spike waveforms in each of the 16 channels, are remarkably constant over the 3 months of recording. There is no evidence of loss of isolated neuron signals over this period, although new neuron signals are observed in 11 out of 16 channels during the first 2 weeks (Fig. 3b,c and Supplementary Fig. 15). These results contrast with the decrease in recorded neurons with time for conventional neural probes34,35, as well as the ~1 month period necessary to first see stabilized and well-isolated single units with mesh electronics17. Second, the number of recorded neurons varies across channels in a way that correlates with the neuron soma density in different HIP subfields.

Third, we have carried out analyses to assess the strength of our above conclusion based on sorted neuron spikes. Principal component analysis35,36 shows the same clusters with relatively constant positions in the first and second principal component plane (PC1–PC2) over the 12 weeks studied (Supplementary Fig. 16), thus demonstrating single-unit stability over time. In addition, L-ratio analysis (Supplementary Note 5)37,38 shows values of <0.05 for all detected neurons (Supplementary Table 3) and indicates minimal contamination between isolated units and thus good unit separation.
Furthermore, quantitative waveform autocorrelation analysis for the same neuron recorded on different days (Supplementary Fig. 17 and Supplementary Note 5)\(^5\) demonstrates that the neuron spikes have consistent characteristic waveform features over time, which is indicative of chronic recording stability.

Finally, we repeated the time-dependent electrophysiology studies and analyses for two additional mice implanted with NeuE probes (Supplementary Figs. 18 and 19). These results are consistent with the data presented in Fig. 3 (and Supplementary Figs. 12–17), with a total of 40 initially connected channels, demonstrating the reproducibility of the stable single neuron recording and tracking of NeuE probes. Furthermore, analyses of mechanical stress in deformed NeuE elements characterized in 3D images of the implanted probes demonstrate that the maximum stress is well below the fatigue failure limit of the SU-8 material\(^39\) used in fabrication (Supplementary Note 6), thus indicating that NeuE probes could exhibit long-term mechanical robustness beyond the 3 months studied.

The combined electrophysiology and imaging data allowed us to ask whether the intact interface between the NeuE electrodes and neurons across the HIP and CTX could allow the spatial coordinates of the same spiking neuron detected by three nearby recording electrodes to be determined by triangulation (Supplementary Note 5)\(^5,40,41\). Triangulation has been used previously\(^40,41\), although it has not been possible to register the electrode–neuron positions at the cellular scale as probes must be extracted for histology studies\(^43,46,61\). An example is that of electrodes 12, 13 and 14, which record the same neuron (Fig. 3d); this was identified (Fig. 3d,II) from the population of CA1 neurons surrounding these electrodes (Fig. 3d.I) by in situ reconstruction of the 3D electrodes–neurons interface. We suggest this capability of mapping recorded cells onto the undisturbed imaged brain structures could open new opportunities to link directly functional and topological circuits within their physiological context. This is distinct from reported methods combining in vivo imaging and subsequent in vitro brain slice electrophysiology, where additional neuron transduction for structural marker expression\(^63\) or fluorescent microspheres\(^43\) are required to re-identify the same neurons. Moreover, this registration-free in situ 3D mapping capability opens the possibility of directly correlating experience-dependent neuronal activity with the underlying neuronal connectivity when combined with recent neuronal activity markers such as immediate early genes\(^41\).

**NeuE promotes the migration of newborn neurons**

The 3D interpenetration of neurons through NeuE probes and the observation of new spiking neuron signals at early times led us to ask whether native neural progenitor cells (NPCs) originating from the subgranular zone (SGZ) of the HIP\(^45,46\) migrate and concurrently develop into newborn neurons along the neurite-like structure of the NeuE since probes are implanted with ends touching or spanning the SGZ (Fig. 4a inset and Supplementary Fig. 20a). The presence of NPC-derived newborn neurons was assessed using staining of DAPI\(^\) for newborn neurons 45,46 and 4’,6-diamidino-2-phenylindole (DAPI) for nuclear DNA, following tissue clearing (see Methods)\(^50,51\). Representative 3D images of brain tissue at 1 week post-implantation (Fig. 4a,b) show substantial DCX\(^+\) cells associated and aligned with the NeuE structure, yet little or no DCX staining of DAPI\(^+\) cells further away from the probe.

To gain further insight into the importance of the NeuE biomimetic structure in promoting the association and migration of DCX\(^+\) newborn neurons, we also assessed NeuE-injected samples collected at 2 weeks post-implantation (Supplementary Fig. 20) and controls using the significantly larger (20 µm width features) mesh electronics (Supplementary Fig. 21). Overall, there is a statistically significant decrease ($P<0.001$) in the enhancement of DCX\(^+\) cells near NeuE at 2 weeks versus 1 week (Fig. 4c, Supplementary Fig. 20 and Supplementary Note 4) and, strikingly, the 20 µm control mesh shows a substantial approximately threefold decrease ($P<0.001$) in DCX\(^+\) cells compared to NeuE at both time points (Fig. 4c, Supplementary Fig. 21 and Supplementary Note 4). These quantitative data suggest that the biomimetic neurite-like topographical features of NeuE may modulate the endogenous NPC-derived cell behaviour including adhesion and migration\(^45\), and help to explain the rapid tissue healing and the emergence of ‘new’ spiking neurons.

**Summary and outlook**

The NeuE represents a strong case for the use of bioinspiration and biomimicry as tools for the design of innovative materials and devices for neurotechnology. In contrast to other state-of-the-art electrophysiology probes12–15,17–20,22,24,34,35, NeuE probes match the subcellular feature sizes and mechanical properties of neurons. NeuE probes elicit negligible immune response compared to other probes12,14,17–20 and exhibit seamless interpenetrating interfaces with the brain from day 2 post-implantation onwards. In addition, NeuE probes demonstrate stable single-unit recording of individual cells in the nearly native physiological context without loss in recording quality from shortly after implantation to at least 3 months. We suggest that these characteristics arise from matching the structural and mechanical properties to the local cellular components within...
the microenvironment, which largely eliminates micromotion and tissue damage associated with signal instability. In addition, NeuE is a substrate for the migration of newborn neurons, suggesting its potential to direct cells towards sites of damage where they could help repair or regenerate damaged tissues, and as a multimodal platform for actively remodelling tissue while simultaneously monitoring such changes. Nevertheless, multifunctional integration that allows for simultaneous optical, electrical and chemical interrogation and high spatial integration that allows for simultaneous recording of a larger number of neurons represent interesting directions to pursue in the future, for example, by integrating higher-resolution fabrication methods. In addition, given that the current NeuE design has virtually eliminated structural and mechanical distinctions with the fundamental neuron component of the brain, we suggest that this can serve as an ideal starting point to exploit biochemical modification strategies to endow it with added functionality. More generally, we believe that the neural interface of bioinspired NeuE, which is structurally and mechanically indistinguishable from neurons, gliosis-free, functionally stable and pro-regenerative, can open opportunities for next-generation brain–machine interfaces and biomedical therapeutics.

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Author contributions
X.Y. and C.M.L. designed the experiments. X.Y., T.Z., T.J.Z., G.H., Y.Z., R.D.V., T.-M.F. and T.G. performed the experiments. X.Y., T.Z., T.J.Z. and C.M.L. analysed the data. X.Y. and C.M.L. wrote the paper. All authors discussed the results, revised or commented on the manuscript.

Competing interests
The authors declare no competing interests.

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Methods

Design and fabrication of NeuE. The design and structural parameters of NeuE are distinct from previous reports17–27, although the overall fabrication approach is similar to our previous reports17–20. The parameters of the two NeuE designs used for these studies are summarized in Supplementary Table 1. Barcodes based on a unique combination of triangles and circles associated with electrodes (Supplementary Fig. 11b) were also introduced for indexing the recording electrodes in electrophysiological experiments. Here we focus on 16-channel NeuE probes, but note that higher channel numbers can be achieved20 without varying the reported designs by using higher resolution fabrication26.

The key fabrication steps for NeuE probes are as follows. (1) A 100-nm-thick Ni sacrificial layer was thermally evaporated (Sharon Vacuum Co.) onto a 3-inch Si wafer (n-type 0.005 Ω·cm, 600 nm thermal oxide, NOVA Electronic Materials), which was pre-cleaned with oxygen plasma (100 W, 5 min; AST Products). (2) Negative photoresist SU-8 (SU-8 2000.5; MicroChem) was mixed with Lissamine rhodamine B sulfonyl dihydrazide (RhBen; 10 mg·μl−1; Thermo Fisher Scientific) and placed in the dark at room temperature (RT) for 3 days. The primary amine groups of RhBen covalently react with the epoxide groups of SU-8 to afford stable fluorescence labeling26. To eliminate any possible undissolved RhBen, the SU-8/RhBen solution was then centrifuged (Thermo Fisher Scientific) at 10,000 r.p.m. for 3 min immediately before use. The supernatant of SU-8/RhBen solution was spin-coated on the Si wafer to a thickness of ∼420 nm, pre-baked sequentially at 65 °C for 1 min and 95 °C for 4 min, and then patterned by photolithography with a mask aligner (SUSS MA6 mask aligner, SUSS MicroTec). After photolithography exposure, the Si wafer was post-baked sequentially at 65 °C for 1 min and 95 °C for 3 min. (3) The SU-8 photoresist was developed with diluted S1805 (1:3 (vol:vol) diluted in Thinner-P, Microchem) for 2 min, rinsed with isopropanol, dried with N2 and baked at 65 °C for 3 min and 95 °C for 3 min. After photolithography exposure, the Si wafer was post-baked sequentially at 65 °C for 1 min and 95 °C for 3 min. (4) The SU-8 photoresist with the patterned SU-8 Developer (MicroChem) for 2 min, rinsed with isopropanol, dried with N2 and hard baked at 180 °C for 1 h. (4) The Si wafer was then treated with oxygen plasma (50 W, 30 s), spin-coated with MCC Primer 80/20 and LOR 3A resist (Microchem) and baked at 180 °C for 5 min. For NeuE design 1, Shipley 1805 positive photoresist (Microposit, Dow Chemical Company) was spin-coated on the Si wafer and baked at 115 °C for 5 min. The positive resist was patterned by photolithography (SUSS MA6 mask aligner) and developed (MF-CD-26, Microposit, Dow Chemical Company) for 90 s. For smaller NeuE design 2, diluted S1805 (1:3 (vol/vol) diluted in Thinner-P, Microchem) was spin-coated on the Si wafer and baked at 115 °C for 1 min. The positive resist was patterned by photolithography with a mask aligner (MLA150) and developed (MF-CD-26) for 30 s. (5) After a flood exposure of the whole wafer, a 3-mm-thick Cr layer and a 100-nm-thick Au layer were sequentially deposited by electron-beam evaporation (Denton Vacuum), followed by a liftoff step (MF-CD-26) for the Au interconnect lines. (6) Steps 4 and 5 were repeated for photolithography patterning and deposition of Pt recording electrodes (3-mm-thick Cr and 50-nm-thick Pt). (7) Steps 2 and 3 were repeated for photolithography patterning of the top SU-8 layer as the encapsulation and insulating layer of the metal interconnect lines. After hard baking the top SU-8 layer at 180 °C for 1 h, the Si wafer was hard baked again at 190–195 °C for 1 h to allow interdiffusion of the bottom and top SU-8 layers to yield an effectively monolithic component. (8) To release NeuE probes, the Si wafer was cleaned with oxygen plasma (50 W, 30 s) and then transferred to a Ni etchant solution comprising 40% FeCl3:39% HCl:H2O (Patterson Veterinary Supply) and 1 mg kg−1 desoximethyl (Orion). The design and structural parameters of NeuE were rendered red and metal was rendered yellow.

In vivo mouse survival surgery. Stereotaxic injection of NeuE in mouse brains. In vivo injection of NeuE probes into live mice was performed using a controlled stereotaxic injection method as described previously26 and reproduced here for completeness. All metal tools in direct contact with the animal subjects were bead-sterilized (Fine Science Tools) for 1 h before use, and all plastic tools in direct contact with the animal subjects were sterilized with 70% ethanol and rinsed with sterile DI water and sterile 1 × PBS before use. The NeuE probes were sterilized with 70% ethanol followed by rinsing in sterile DI water and sterile 1 × PBS before injection, and then loaded into sterile glass capillaries with an inner diameter of 400 μm and outer diameter of 550 μm (Prodistral). Mice were anesthetized by intraperitoneal injection of a mixture of 75 mg kg−1 (body weight) of ketamine (Patterson Veterinary Supply) and 1 mg kg−1 desoximethyl (Orion). The minute of anesthesia was verified via toe pinch before surgery started. A homeothermic blanket (Harveto 37 °C) was placed next to the anesthetized mouse; the anesthetized mouse was placed in a stereotaxic frame (Lab Standard Stereotactic Instrument, Stoelting Co.) equipped with two ear bars and one nose clamp. Puralube vet ointment (Dechra Pharmaceuticals) was applied on both eyes to moisturize the eye surface throughout the operation. Hair removal lotion (Nair, Church & Dwight) was applied to the scalp for depilation and Betadine surgical scrub (Purdue Products) was applied to the skin. A 1 × PBS solution of 10% sodium hypochlorite (2% bleach) was used to wipe the scalp skin. A sterile scalpel was used to make a 5 mm longitudinal incision in the scalp along the sagittal sinus. The scalp skin was resected to expose a 5 × 5 mm portion of the skull.

In the surgeries for electrophysiological recording, Metabond adhesive luting cement (Parkell) was applied over the exposed cranial bone to prepare the skull surface for mounting the electronics later. A 1 mm burr hole was drilled using a dental drill (Micromotor with On/Off Pedal 110/220, Grobet USA) with the following stereotaxic coordinates: anteroposterior, −4.96 mm; mediolateral, 3.10 mm. The dura was carefully incised and resected using a sterile 27-gauge needle (PrecisionGlide, Becton Dickinson), then a sterile 0–80 set screw (18–8 stainless steel cup point set screw, outer diameter 1.52 mm, groove diameter 1.14 mm, length 4.76 mm; McMaster-Carr Supply Company) was screwed into this 1 mm burr hole to a depth of 500 μm to serve as the grounding and reference electrode. Metabond adhesive luting cement was used to fix the junction between the screw and the skull.

A 1-mm-diameter burr hole was drilled for the injection of NeuE according to the following stereotaxic coordinates: anteroposterior, −2.30 mm; mediolateral, 1.75 mm; dorsoventral, 2.00 mm. The dura was removed from the burr hole, and a 1 × PBS was swabbed on the skull surface to keep it moist throughout the surgery. NeuE was injected into the targeted brain region using the controlled injector method reported previously26. In brief, the glass micropipette needle loaded with sterilized NeuE was inserted in the micropipette holder, which was connected to a 5 ml syringe (Becton Dickinson), which was pre-filled with 1 × PBS and mounted on a syringe pump (PHD 2000, Harvard Apparatus). The micropipette holder was mounted on a stereotaxic stage equipped with a motorized linear translation stage (860A motorizer and 460A linear stage, Newport) that could move the stereotactic arm in the z direction with a constant preset velocity ranging from 0.05 to 0.5 mm s−1. The needle was positioned at the surface of exposed brain tissue and then inserted into the brain tissue to the desired coordinates, which, when coupled with a rational design of electrode distribution, allows for the placement of electrodes at particular locations within the brain. Controllable injections were carried out by syringing the syringe pump at a motorized linear translation stage, with a typical liquid injection rate of 5–15 μl h−1 and a typical translational stage retraction velocity of 0.2–0.5 mm s−1. The volumetric flow rate and needle retraction velocity were adjusted such that the upper part of NeuE, which was visualized through an eyepiece camera (DCC1240C, Thorlabs), remained stationary in the field of view. The total injection volume was usually 5–50 μl over the 2 mm length of injection. Control experiments with sham injections were performed in a similar manner without NeuE implantation, but with the injection of 30 μl of 1 × PBS. Additional details of the implantation process are available at http://meselectronics.org/protocols.html#injection.

**Safety closure for 3D mapping surgeries.** After implantation of the NeuE, the scalp was closed with 3M Vetbond tissue adhesive. Triple antibiotic ointment (WATER-JEL Technologies) was applied copiously on the closure.

**Electrical connection of NeuE for chronic recordings.** After injection of the NeuE into the targeted region of the mouse brain, the glass capillary was repositioned over

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**Vertebrate animal subjects.** Adult (6–8 weeks, 25–35 g) male B6.Cg-Tg(Thy1-YFP)Hjerfsijl (Thy1-YFP, stock no. 003782, The Jackson Laboratory), B6.Cg-Tg(Thy1-YFP)16fs1 (Thy1-YFP, stock no. 003709, The Jackson Laboratory), FVB/N-Tg(GFAPGFP)14Melsj (GFAP-GFP, stock no. 003257, The Jackson Laboratory) and C57BL/6J mice (stock no. 000664, The Jackson Laboratory) were used in the study. Exclusion criteria were pre-established: animals with substantial acute implantation damage (>50% of initial liquid injection volume) or damage of a major blood vessel were discarded from further experiments.

All procedures were approved by the Animal Care and Use Committee of Harvard University. The animal protocols used in this study were approved by the Harvard University IACUC as well as by the Institutional Animal Care and Use Committee at Harvard University. The research was performed in compliance with the guidelines for the care and use of laboratory animals of the American Association for Accreditation of Laboratory Animal Care (AAALAC). Before surgical procedures, animals were group-housed on a 12 h:12 h light:dark schedule in the Harvard University Biomedical Research Infrastructure (BRID) and fed with food and water ad libitum as appropriate. Animals were housed individually after surgical procedures.

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a flexible flat cable (FFC, Premeo-Flex, Molex), and then the remaining part of the NeuE was expelled from the capillary and unfolded onto the FFC to expose the input–output connection pads. Bonding of the NeuE input–output pads to the FFC was carried out by a previously reported conductive ink printing method. In brief, a carbon nanotube solution (stock no. P093099-11, Tubes@Rice) was loaded into a pulled glass capillary printer head with an inner diameter of ~150 μm by capillary forces. The printer head was fixed to a holder (Warner Instruments) and then mounted onto a motorized micromanipulator (MP-285/M, Sutter Instruments), controlled by a rotary optical encoder (NPC-200, Sutter Instruments) and controller (MPC-200, Sutter Instrument). A custom-written LabVIEW program (available at http://meshelaboratories.com/static/ctl-labview.zip) was used to set the starting and ending positions for each channel and drove the printer head to print the conductive ink in between automatically. Each channel of the NeuE could be individually connected to the corresponding channel on the FFC. The entire FFC with capillaries nanotube-bonded NeuE was then used by applying more Metabond quick adhesive luting cement on the top and side of the FFC and the exposed mouse skull while the other end of the FFC was uncovered and was connected to the recording system via a custom printed circuit board interface board. Recently, a simplified plug-and-play input–output connection methodology has been reported. This strategy could be adapted to the NeuE design without affecting the crucial bioinspired implanted region of the NeuE design.

Postoperative care. After surgery, each mouse was returned to a cage equipped with a 37 °C heating pad. The activity of the mouse was monitored every hour until it was fully recovered from anesthesia. Rennelin (Buprenorphine, Patterson Veterinary Supply) analgesia was given intraperitoneally at a dose of 0.05 mg/kg body weight every 12 h for up to 72 h post-surgery.

3D mapping of the NeuE–brain interfaces. Histology sample preparation. Mice with implanted NeuE were anesthetized with ketamine/xedemorid and transcardially perfused with ice-cold 40% 1× PBS and 40% 4% formaldehyde (Electron Microscopy Sciences) at specified times post-injection, followed by decapitation. The scalp skin was removed and the exposed skull was ground for 10–20 min at 10,000 r.p.m. using a high-speed rotary tool (Dremel). Brains with the NeuE undisturbed were removed from the cranium and placed in 4% formaldehyde for 24 h and then transferred to 1× PBS (Sigma-Aldrich) at 37 °C for 10–20 min at 10,000 r.p.m. using a high-speed rotary tool (Dremel). Brains were then embedded in 3% agarose (Electron Microscopy Sciences) at specified times post-injection, followed by removing any remaining formaldehyde. Brains were then sectioned with a 37 °C heating pad. The activity of the mouse was monitored every hour until it was fully recovered from anesthesia. Rennelin (Buprenorphine, Patterson Veterinary Supply) analgesia was given intraperitoneally at a dose of 0.05 mg/kg body weight every 12 h for up to 72 h post-surgery.

Microscopy imaging. Fluorescence images were acquired on a Zeiss LSM 880 confocal/multiphoton microscope (Carl Zeiss Microscopy) with a ×20 objective (numerical aperture 1.0, free working distance 5.6 mm). Near-infrared lasers were used for two-photon excitation of endogenously expressed fluorescent proteins in the transgenic mouse brain slices. Specifically, an 840 nm laser was used to excite 2P-YFP, a 950 nm laser to excite YFP and a 920 nm laser to excite GFP. The bandpass filter for RhIB was 575–610 nm and that for YFP and GFP was 500–550 nm. Confocal images of cleared and immunostained brain slices were acquired using 405, 488, 561 and or 633 nm lasers as the excitation sources for DAPI, Alexa Fluor 488, RhIB/Alexa Fluor 594 and or Alexa Fluor 647, respectively. The corresponding bandpass filters were diaphragms 411–496, 500–553, 562–624, 624–660 and or 677–755 nm, respectively. Images were acquired by taking a tile scan together with Z stacks, with a voxel size of 0.2–0.6 μm (x) × 0.2–0.6 μm (y) × 0.4–2.5 μm (z) and tile scan overlap of 10%. YFP, GFP (GFAP), Iba-1, DCX, DAPI and NeuE are represented as green, cyan, magenta, yellow, blue and red in the composite images.

Chronic electrophysiological recording from awake and restrained mice. The following procedures were performed according to previous works and are reproduced here for completeness. The electrophysiology of mice with implanted NeuE was recorded every day for ~30 min during the first half week post-implantation, every other day until the second week, followed by twice a week thereafter. Mice were restrained in a Talreis restrainer (Braintree Scientific) while their head-mounted FFCs were connected to an Intan RHD 2132 amplifier evaluation system (Intan Technologies) through a homemade printed circuit board. 0–80 set screws were used as references. Electrophysiological recordings were acquired with a 20kHz sampling rate and a 60 Hz notch filter, while the electrical activity of each recording electrode at 1 kHz was also measured by the same system and showed consistent values of <1 ΜΩ that allow for single unit recording measurements (Supplementary Fig. 14a). Thy1-YFP mice were usually recorded for 3 months until they were transcardially perfused and underwent the same histology sample preparation procedure as described in the above section ‘3D mapping of the NeuE–brain interfaces’.

Reporting summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Code availability
The code used for data analysis is available from the corresponding author upon reasonable request.

Data availability
The data that support the findings of this study are available from the corresponding author upon reasonable request.

References
Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

- n/a Confirmed
- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
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  - Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
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- A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  - Give P values as exact values whenever possible.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection
- ZEN 2.1 software was used to acquire fluorescence images. Intan RHD2000 system was used to acquire electrophysiology data. Inspect-X software was used to acquire micro-CT X-ray data and CT Pro 3D software was used to reconstruct the images. No custom software or code was used for data collection.

Data analysis
- Imaris 9, ImageJ, WaveClus 2.0 and custom MATLAB code were used for histology and electrophysiology data analysis. VG Studio MAX 3.0 software was used to render and analyze the micro-CT 3D reconstructed images.

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data that support the findings of this study are included in the paper and the Supplementary Information. Further details are available from the corresponding author upon reasonable request.
Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size
Sample sizes were selected to achieve at least 80% power at a significance level of 0.05. The sample size for each experiment is detailed in the Supplementary Table 2.

Data exclusions
Exclusion criteria were pre-established: animals with failed surgeries or substantial acute implantation damage (>50 μL of initial liquid injection volume) were discarded from further experiments.

Replication
Replicate experiments on independent animals were performed to ensure the results are reproducible. Number of replicates for each experiment is detailed in the Replication section of Supplementary Information and Supplementary Table 2. Attempts at replication were successful and the conclusions were drawn from the analysis of multiple experiments.

Randomization
Mice were randomized into different experimental groups.

Blinding
Blinding was used during fluorescence image acquisition.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

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Methods

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<td>MRI-based neuroimaging</td>
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Antibodies

Primary antibodies:
- Rabbit anti-doublecortin (Abcam, Catalog # ab18723, Lot # gr3224908-1);
- Rat anti-GFAP (Thermo Fisher Scientific Inc., Catalog # 13-0300, Lot # SF255147);
- Goat anti-Iba1 (Abcam, Catalog # ab5076, Lot # gr359324-1).

Secondary antibodies:
- Goat anti-rabbit Alexa Fluor 488 (Abcam, Catalog # ab150077, Lot # gr3200000-1);
- Goat anti-rabbit Alexa Fluor 647 (Abcam, Catalog # ab150079, Lot # gr3176223-3);
- Donkey anti-rabbit Alexa Fluor 488 (Abcam, Catalog # ab130073, Lot # gr3191541-1);
- Donkey anti-rat Alexa Fluor 647 (Abcam, Catalog # ab150079, Lot # gr3183307-2);
- Donkey anti-goat Alexa Fluor 594 (Abcam, Catalog # ab150132, Lot # gr308670-2).

All antibodies were diluted 1:100-1:200.

Validation

All the primary antibodies used in this study have been validated for immunohistochemistry on mouse brain tissues by the supplier and the customers including relevant citations as shown on the supplier’s website. Our data also validate their use on cleared mouse brain tissues.

Animals and other organisms

Policy information about studies involving animals: ARRIVE guidelines recommended for reporting animal research

Laboratory animals

6-8 week male B6.Cg-Tg(Thy1-YFP)H1rs/J [Thy1-YFP-H, stock number 003782, Jackson Laboratory], B6.Cg-Tg(Thy1-YFP)16Irs/J
## Laboratory animals

(Thy1-YFP.16, stock number 003709, Jackson Laboratory), FVB/N-Tg(GFAP-GFP)14Mes1/J (GFAP-GFP, stock number 003257, Jackson Laboratory) and C57BL/6J mice (stock number 000664, Jackson Laboratory) were used in this study.

## Wild animals

The study did not involve wild animals.

## Field-collected samples

The study did not involve samples collected from the field.

## Ethics oversight

All procedures were approved by the Animal Care and Use Committee of Harvard University. The animal care and use programs at Harvard University meet the requirements of the Federal Law (89-544 and 91-579) and NIH regulations and are also accredited by the American Association for Accreditation of Laboratory Animal Care (AAALAC).

Note that full information on the approval of the study protocol must also be provided in the manuscript.