ADVANCED FUNCTIONAL MATERIALS

Supporting Information

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Scalable Thousand Channel Penetrating Microneedle Arrays on Flex for Multimodal and Large Area Coverage BrainMachine Interfaces

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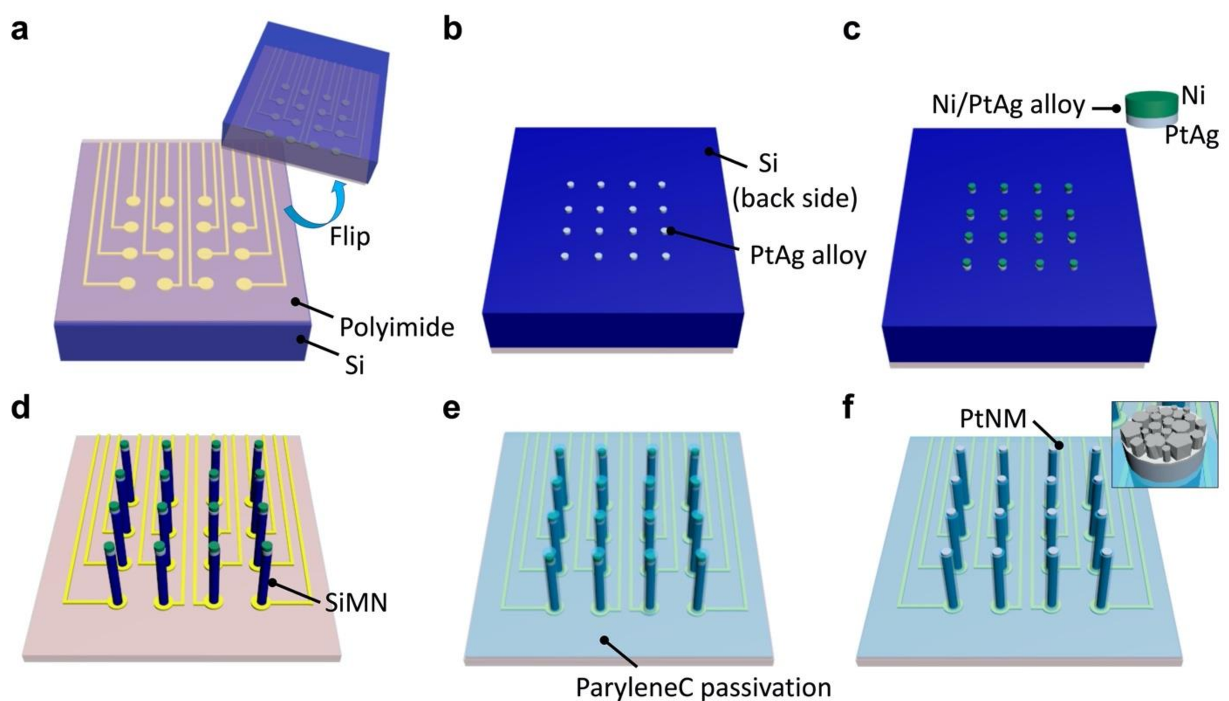


Figure S1. Fabrication process flow of 32-channel SiMNA on flex. **a**, Polyimide layers (10 µm total thickness; 5 µm below and above metal leads) and metal lead stack (electron-beam evaporate 20 nm Ti, 80 nm Ni, 10 nm Cr, and then sputter 250 nm Ti) deposited on a 100 µm-thick, heavily doped n-type Si wafer. Inset shows the wafer flipped view for back-side alignment. **b**, Back-side alignment photolithography and sputter 15 nm Cr, 100 nm Pt, and co-sputter ~0.5 µm of Pt and Ag (PtAg alloy). **c**, Electron-beam evaporate 10 nm Ti and 50 nm Ni. **d**, DRIE (Bosch process) to form SiMN array structure. **e**, Deposit 1 µm parylene-C layer. **f**, Selectively dry etch the SiMN tips and then wet etch Ni with Ni etchant, Ti with BOE and then Ag in the PtAg alloy with HNO_3 to form PtNM structure.

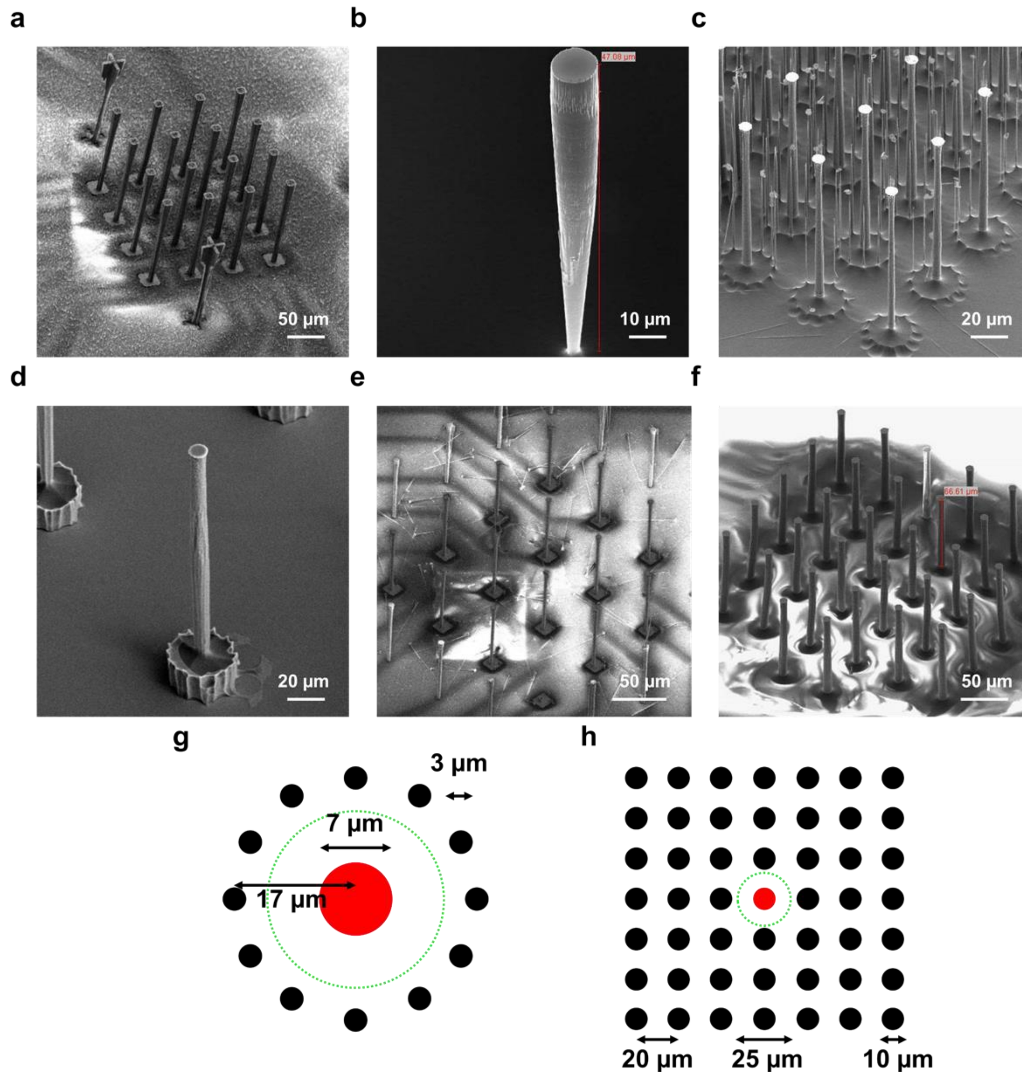


Figure S2. Optimization of 100 µm-tall Si microwire etch. a, SEM image (xx degree view) of prototype device using continuous RIE/ICP etch results in tapering of microwire with the base thinner than the tip. **b**, SEM image (45 degree view) after 2-step DRIE (Bosch etch) on a single Si microwire with undesired tapering similar to **a**. **c**, SEM image (45 degree view) after continuous RIE/ICP etch on a test SiMN array with sacrificial microwires around each main microwire which showed over etch underneath the Ni etch mask at the tip. **d**, SEM image (45 degree view) after 2-step DRIE on a test SiMN array with sacrificial microwires prevents over etch but leaves a wider, elevated base. **e**, SEM image (45 degree view) after additional 2-step DRIE to remove sacrificial microwires leaves undesired residue on the exposed polyimide substrate and thins the main microwires further. **f**, Adequate 2-step DRIE followed by breaking off sacrificial microwires with the probe tip of the micromanipulator setup returns the most desired array morphology. **g**, Top-view of etch mask design of a single microwire (red) surrounded by 12 sacrificial microwires (black), for a 50 µm microwire-to-microwire spacing array. **h**, Top-view of etch mask design of a single microwire (red) surrounded by 48 sacrificial microwires (black), for a 400 µm microwire-to-microwire spacing array. **g-h**, Green dotted lines indicate the boundary of the via at the base of the microwire, at which

the microwire forms an ohmic contact with the exposed metal layer. Outside the green circle is passivated with polyimide substrate.

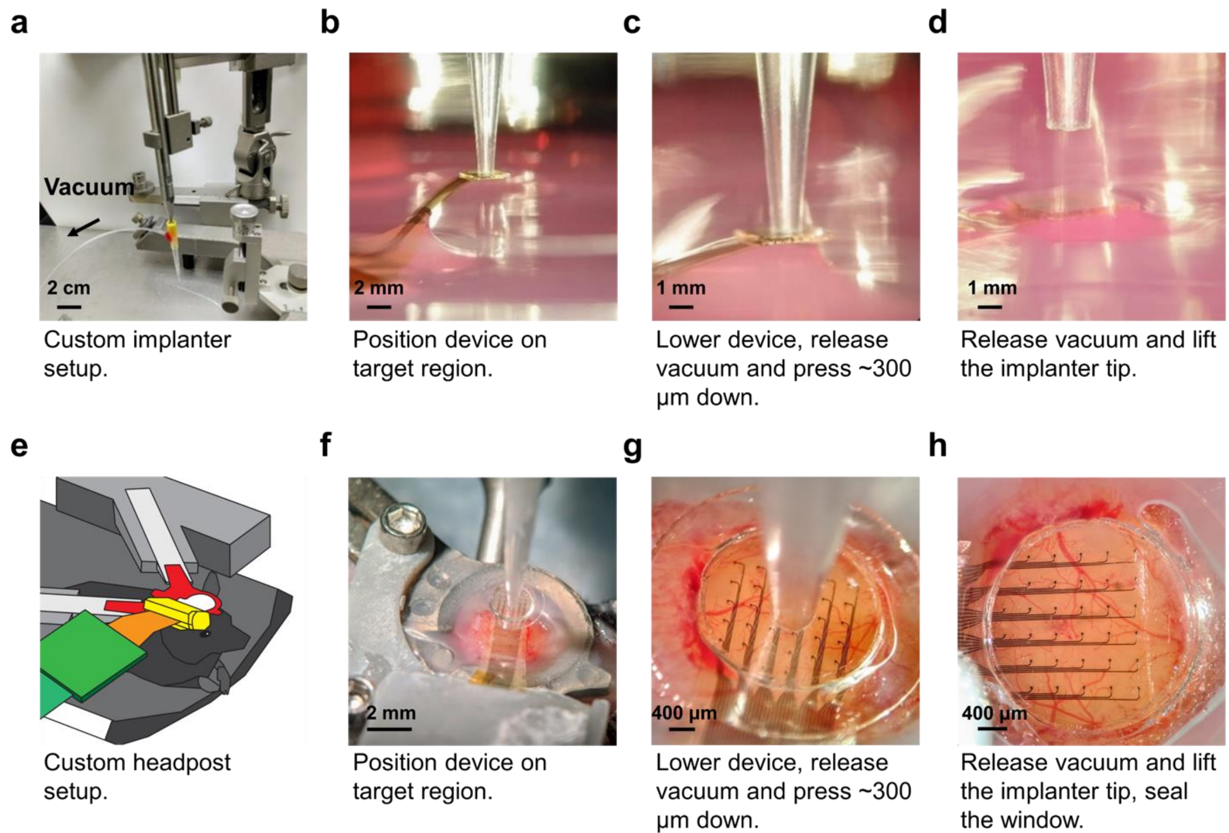


Figure S3. Custom implanter and headpost setup, and optimization of implantation procedure. **a**, A photograph of custom implanter setup. The vacuum-enabled microtip is mounted to a three-dimensional micromanipulator on the stereotaxic instrument. **b-d**, Implantation optimization on agarose test bed. **b**, Vacuum hold the chronic cranial window glued to the backside of the SiMN array with the SiMNs facing downwards. **c**, Wet the target region with artificial cerebrospinal fluid (aCSF), aim and lower using the micromanipulator, and press down additional ~300 µm from the surface. **d**, Release the vacuum and lift the implanter tip. **e**, A schematic of the custom headpost setup showing custom titanium head frame (red) supporting custom 3D-printed headpost (yellow), and PCB connector (green) leading to the recording system. **f-h**, Implantation procedure on mouse somatosensory cortex. **f**, Vacuum hold the chronic cranial window glued to the backside of the SiMN array with the SiMNs facing toward the surface of the mouse brain. **g**, Wet the target region with aCSF, aim and lower using the micromanipulator, and press down additional ~300 µm from the brain surface. **h**, Release the vacuum, lift the implanter tip, and seal the glass window with UV-curable dental cement.

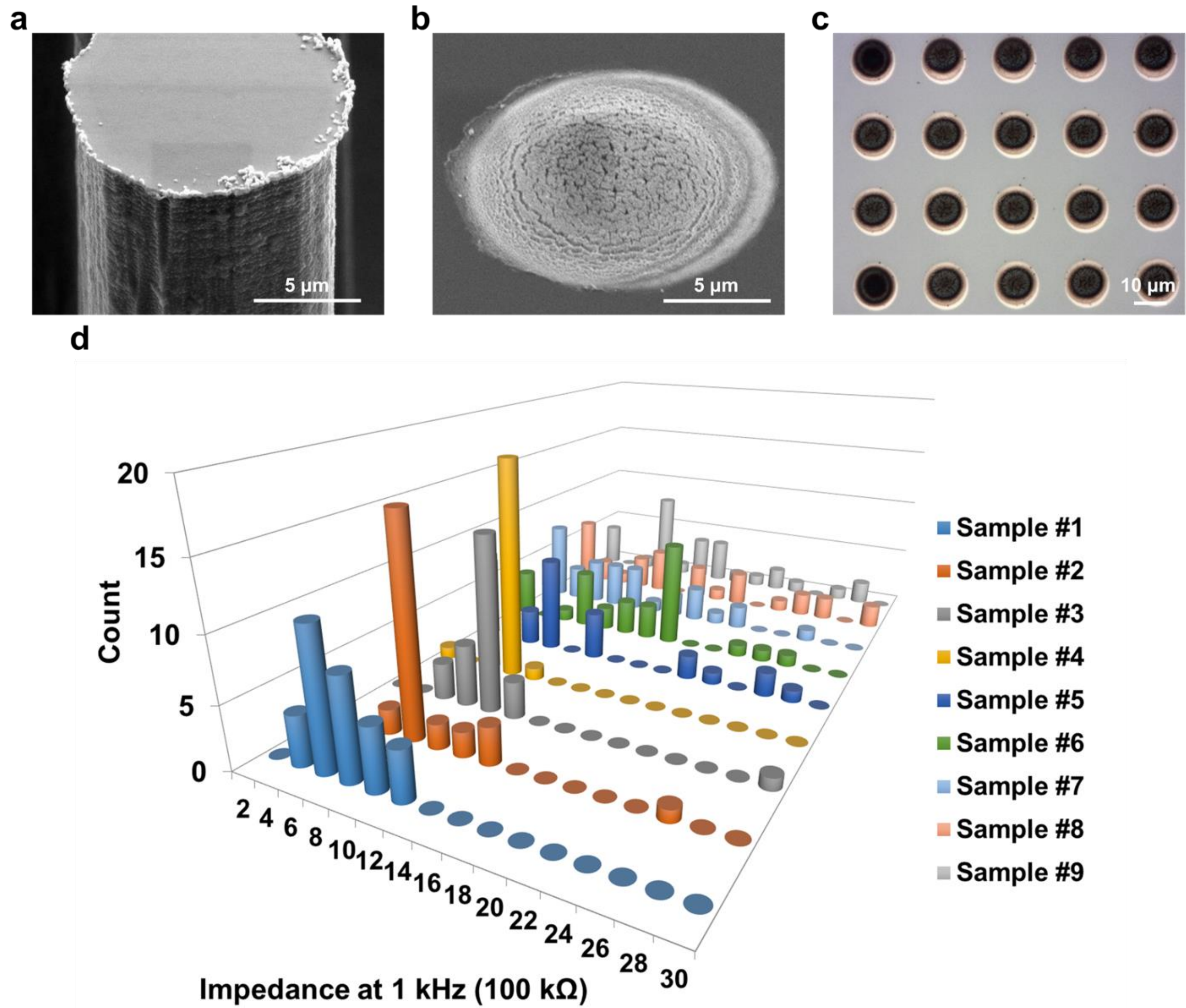


Figure S4. Morphological and electrochemical characterization of PtNM as the sensing electrode material for the SiMN on MNA on flex. **a**, SEM image (45 degree view) of a SiMN with bare Si surface after DRIE (Bosch process). **b**, SEM image (45 degree view) of a PtNM dot. **c**, A top-view magnified photograph of an array of PtNM dots after wet etching Ag from the original PtAg alloy. **d**, A histogram of impedance at 1 kHz for 9 different samples of 32-channel SiMNA on flex with PtNM as the sensing electrode material.

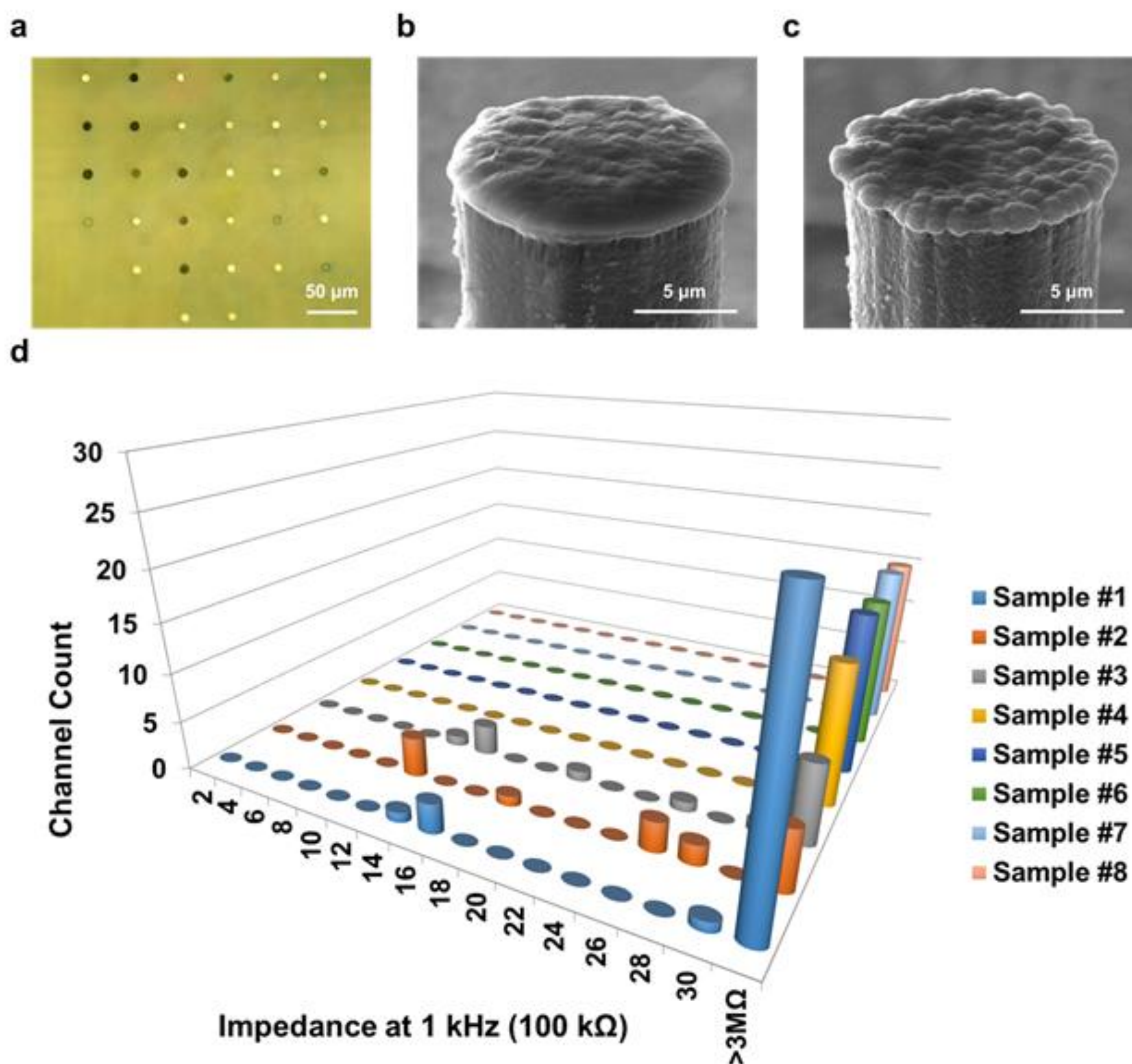


Figure S5. Morphological and electrochemical characterization of PEDOT:PSS as the sensing electrode material for the SiMN on MNA on flex. a, A top-view magnified photograph of an array of electrodeposited PEDOT:PSS at the tip of the SiMN array (black dots are SiMN tips coated with PEDOT:PSS, white dots are SiMN tips with unsuccessful coating of PEDOT:PSS, intermediate colored dots are only partially coated with PEDOT:PSS). **b**, SEM image (45 degree view) of SiMN tip after galvanostatic electrodeposition of PEDOT:PSS. **c**, SEM image (45 degree view) of SiMN tip after potentiostatic electrodeposition of PEDOT:PSS. **d**, A histogram of impedance at 1 kHz for 8 different samples of 32-channel SiMNA on flex with electrodeposited PEDOT:PSS as the sensing electrode material.

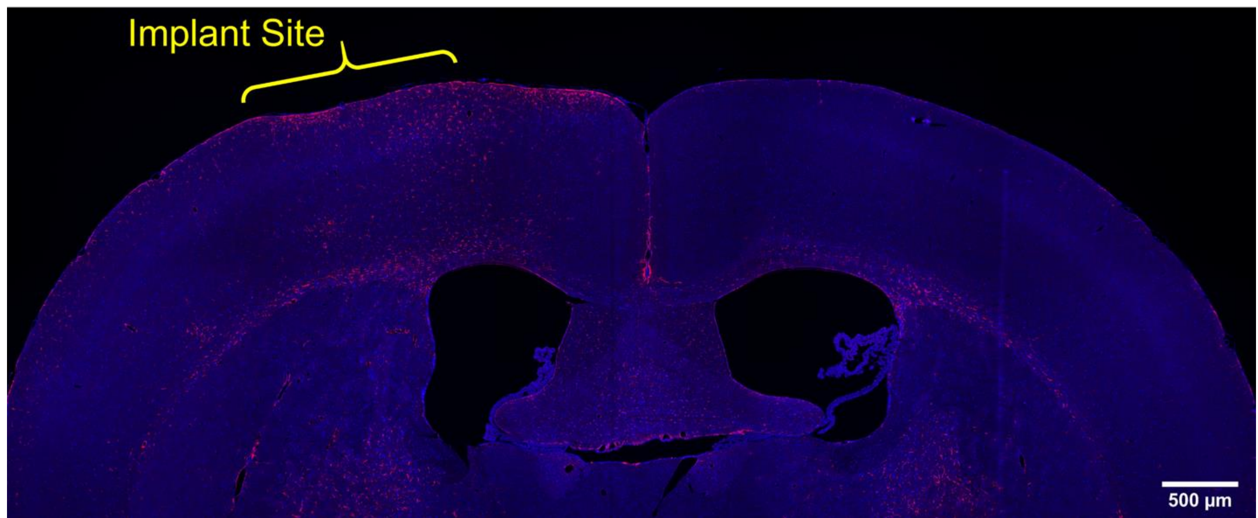


Figure S6. Immunohistochemistry of chronic implantation of SiMNA on Flex. Fluorescent image of DAPI (blue) and GFAP (red) stained coronal tissue section showing the SiMNA-implanted left hemisphere, and right (control) hemisphere after 73 days *in vivo*. Note the highlighted implant site with a dent from the cranial window. The region around the implant site exhibited an increase in astrocytes on par with that observed with cranial openings without implants.

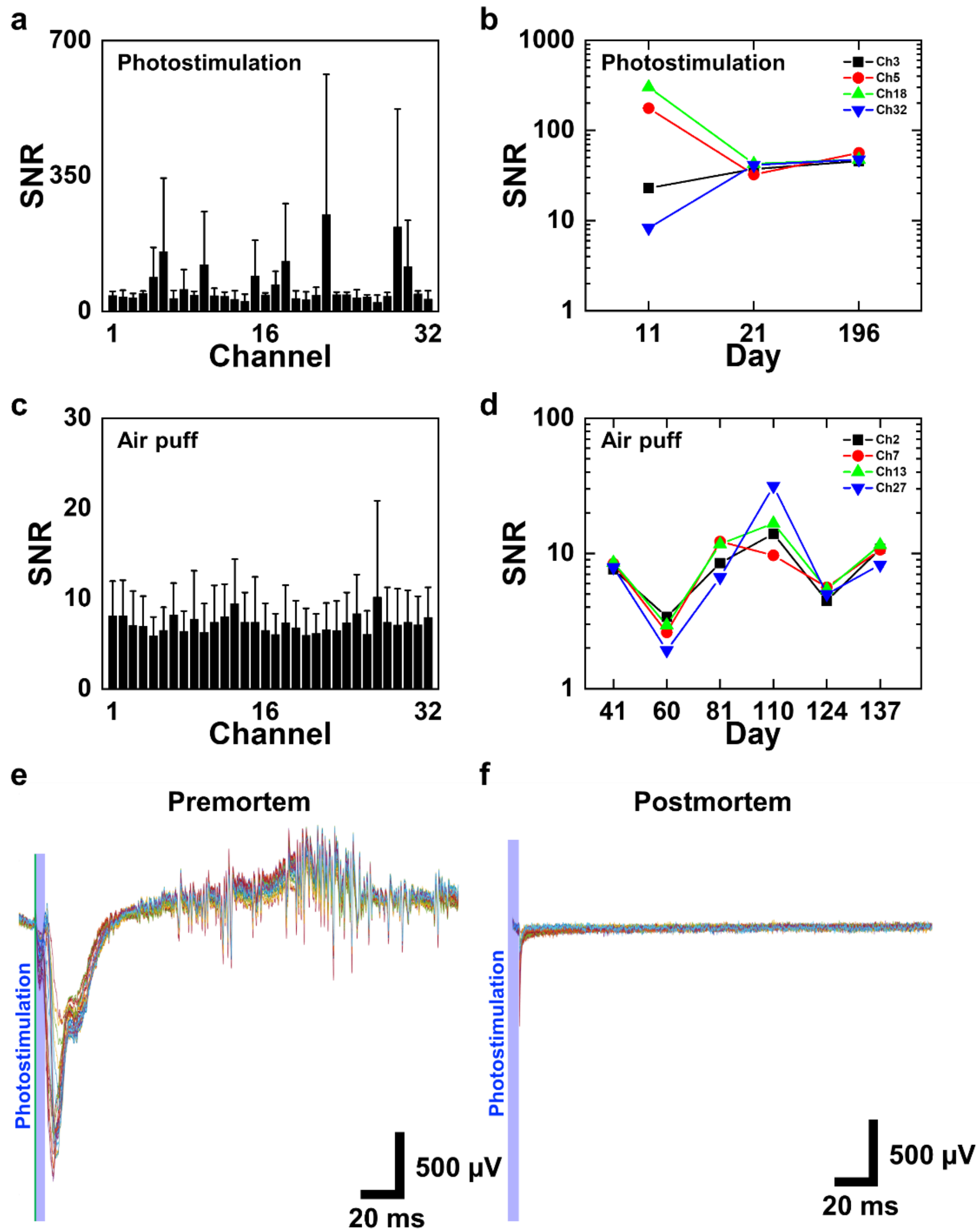


Figure S7. Statistical analysis, and premortem to postmortem comparison of *in vivo* optogenetic photostimulation-evoked responses of a single SiMNA implantation model. a, Histogram of average SNR and standard deviation of 18.7 mW photostimulation-evoked responses (unfiltered) for 32 channels (up to 196 DIV). **b**, SNR change of four channels in the center of the SiMNA. **c**, Histogram of average SNR and standard deviation of air puff stimulation-evoked responses (unfiltered) for 32 channels from the same model as in **a** and **b** (up to 137 DIV). **d**, SNR change of four representative channels from the same model as in **a** and **b**. **e**, Average (N=30) unfiltered premortem optogenetic photostimulation-evoked raw electrical potentials from all channels of a 400 µm-spacing 32-channel SiMNA at 196 DIV. **f**, Average (N=30) unfiltered postmortem optogenetic photostimulation-evoked raw electrical potentials from all channels of a 400 µm-spacing 32-channel SiMNA at 196 DIV. **e-f**, Blue boxes indicate the 18.7 mW optogenetic photostimulation set for duration of 5 ms.

a

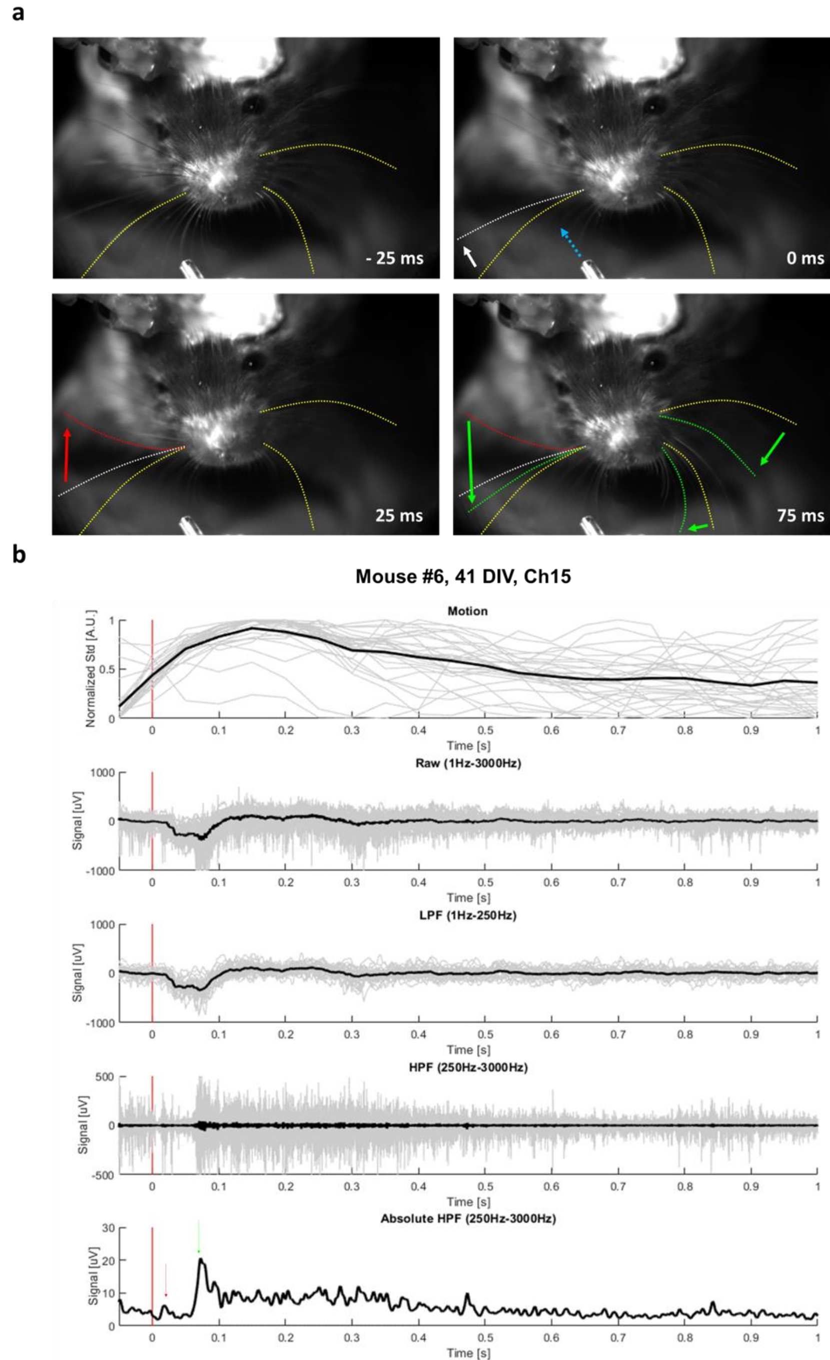


Figure S8. Video recording data for motion detection of whiskers. a, Video footage of the awake mouse in response to air puff stimulation directed towards right side whiskers at 0 ms. Air puff stimulation (blue dotted arrow), the original position of the whiskers (yellow dotted lines) before stimulation, early-stage location of the whisker pushed back by air puff (white dotted line), later stage location of the whisker pushed back by air puff (red dotted line), and initiation of bilateral whisking activity (green dotted lines). **b**, Average detected motion in the mystacial region compared to average waveforms of the filtered electrical recording (N=20). The red arrow indicates the sensory response to the air puff around 25 ms after the stimulus onset (we had ~15 ms delay from the stimulus trigger to the air puff reaching the mouse whiskers), and the green arrow indicates the initiation of whisking activity around 70-75 ms.

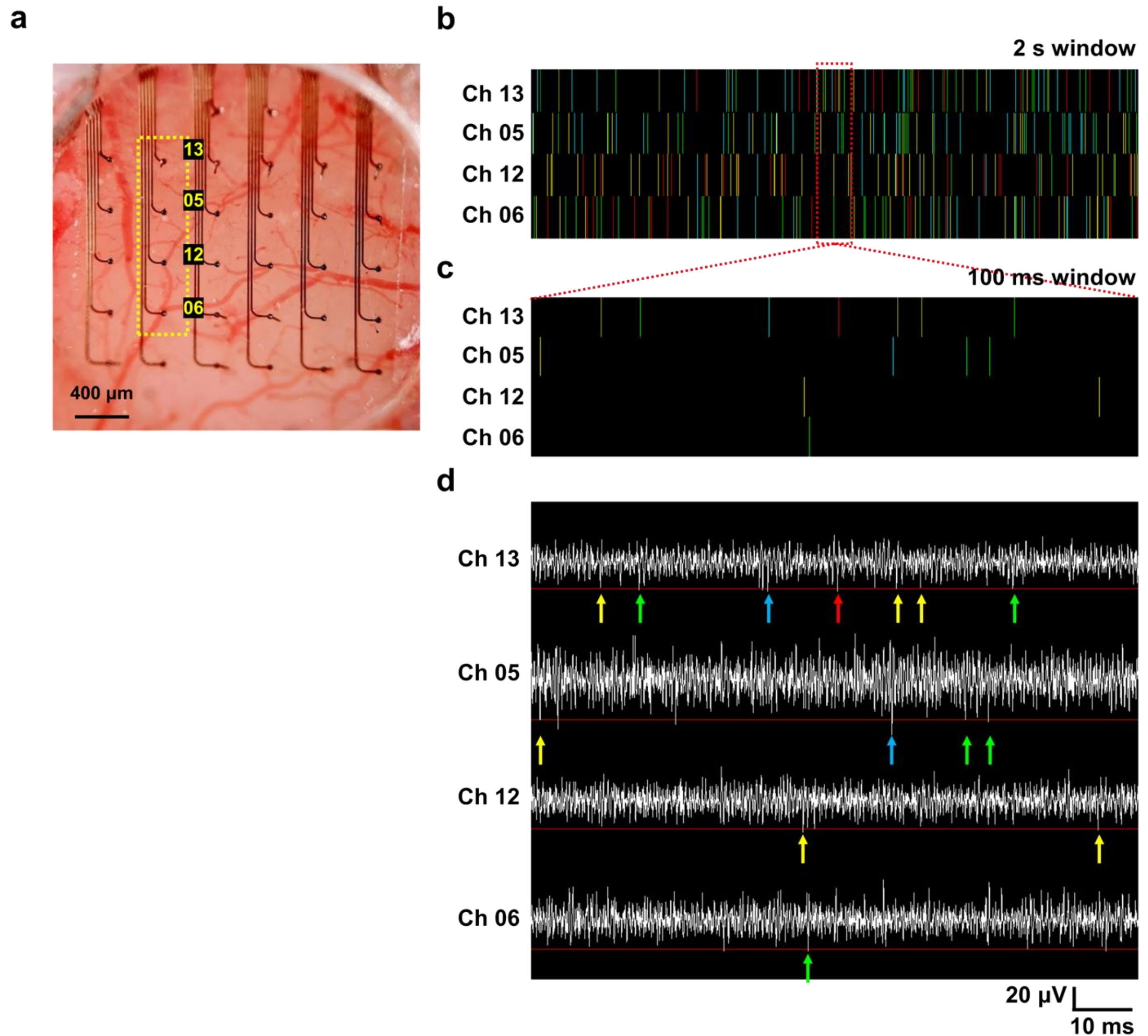


Figure S9. MUA detection. a, A magnified photograph of a 32-channel SiMNA on flex with 400 µm needle-to-needle spacing implanted in mouse #6 somatosensory cortex. Spike sorting was performed on Ch13, 5, 12, and 6 which are highlighted in yellow box. **b**, A spike raster of 2 second window from Ch13, 5, 12, and 6. **c**, A magnified spike raster of 100 millisecond window from Ch13, 5, 12, and 6 in the red highlighted box from **b**. **d**, Filtered and spike detected waveforms from respective thresholds (red line), time-aligned with the spike raster in **c**. Yellow, green, blue, and red arrows indicate time stamps of detected units. **b-d**, Mouse #6, 41 days *in vivo* (Filtered 250-3000 Hz and transferred to Plexon Offline Sorter using custom MATLAB code; Plexon Offline Sorter: waveform length of 1500 µs, pre-threshold period of 350 µs, dead time of 1150 µs, threshold set to -3 times standard deviations from mean of peak heights histogram, automatic sorting using valley seeking scan mode).

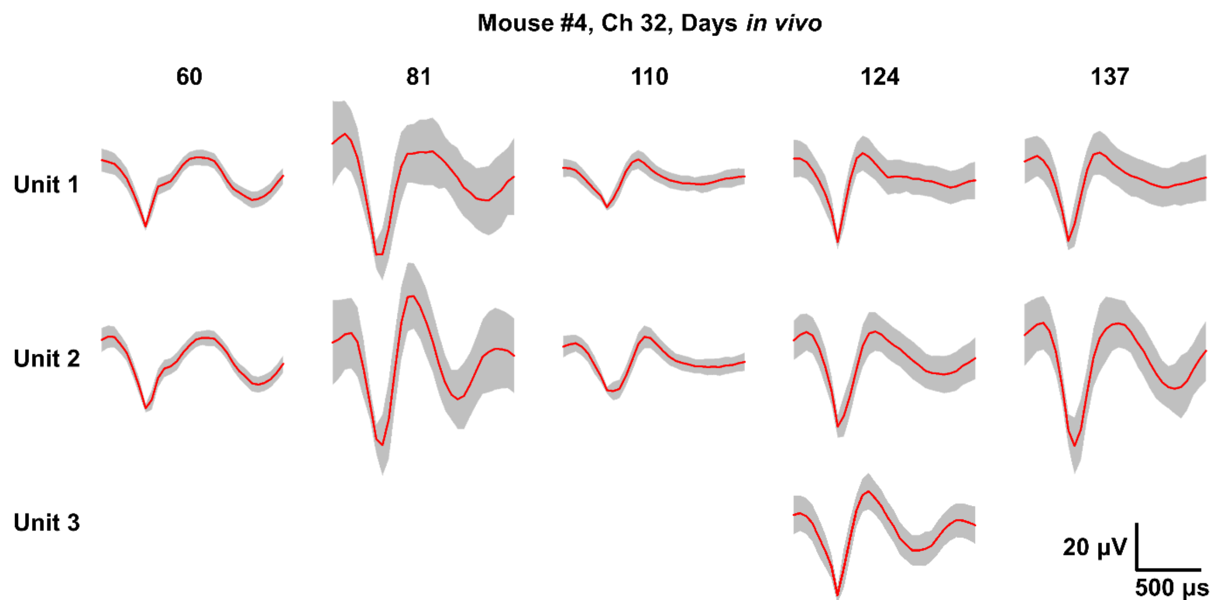


Figure S10. Chronic SUA detection. Waveform of all detected units from recording days between 60 days *in vivo* and 137 days *in vivo* from Ch32 of SiMNA implanted on mouse #4 (filtered 250-3000 Hz and transferred to Plexon Offline Sorter using custom MATLAB code; Plexon Offline Sorter: waveform length of 1500 µs, pre-threshold period of 350 µs, dead time of 1150 µs, threshold set to -3 times standard deviations from mean of peak heights histogram, automatic sorting using valley seeking scan mode).

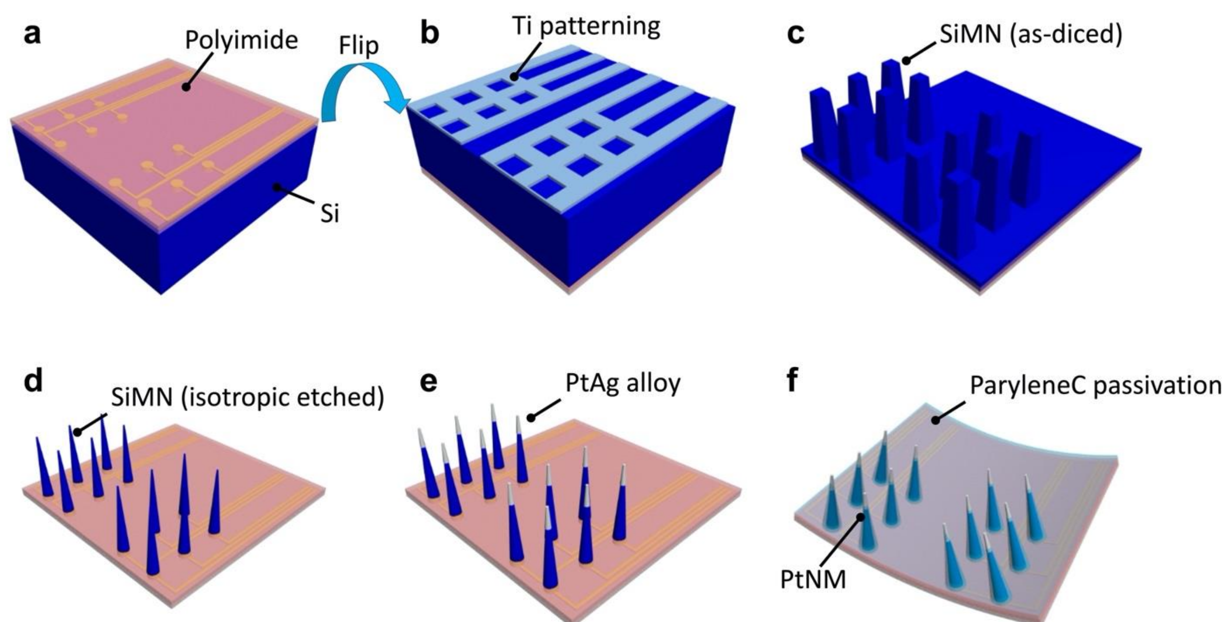


Figure S11. Fabrication process flow of 1024-channel SiMNA on flex. **a**, Polyimide layers (10 µm total thickness; 5 µm below and above metal leads) and metal lead stack (sputter 15 nm Cr and 100 nm Pt) deposited on 500 µm-thick heavily doped n-type Si wafer. **b**, Flip the wafer, back-side alignment photolithography and electron-beam evaporate 50 nm Ti. **c**, Dicing saw creates ~300 µm wide, ~470 µm deep trenches between SiMNs. **d**, Isotropic dry etch using XeF_2 to thin down the SiMNs, followed by DRIE (Bosch process) to remove Si at the bottom to expose the underlying polyimide substrate. Then wet etch with etch solution ($HF:HNO_3$ = 1:19) to sharpen the SiMN tips. **e**, Selectively sputter 15 nm Cr, 100 nm Pt, and selectively cosputter ~0.5 µm of Pt and Ag (PtAg alloy). **f**, Deposit 2 µm parylene-C layer and selectively dry etch the SiMN tips and then wet etch Ag in the PtAg alloy to form PtNM structure.

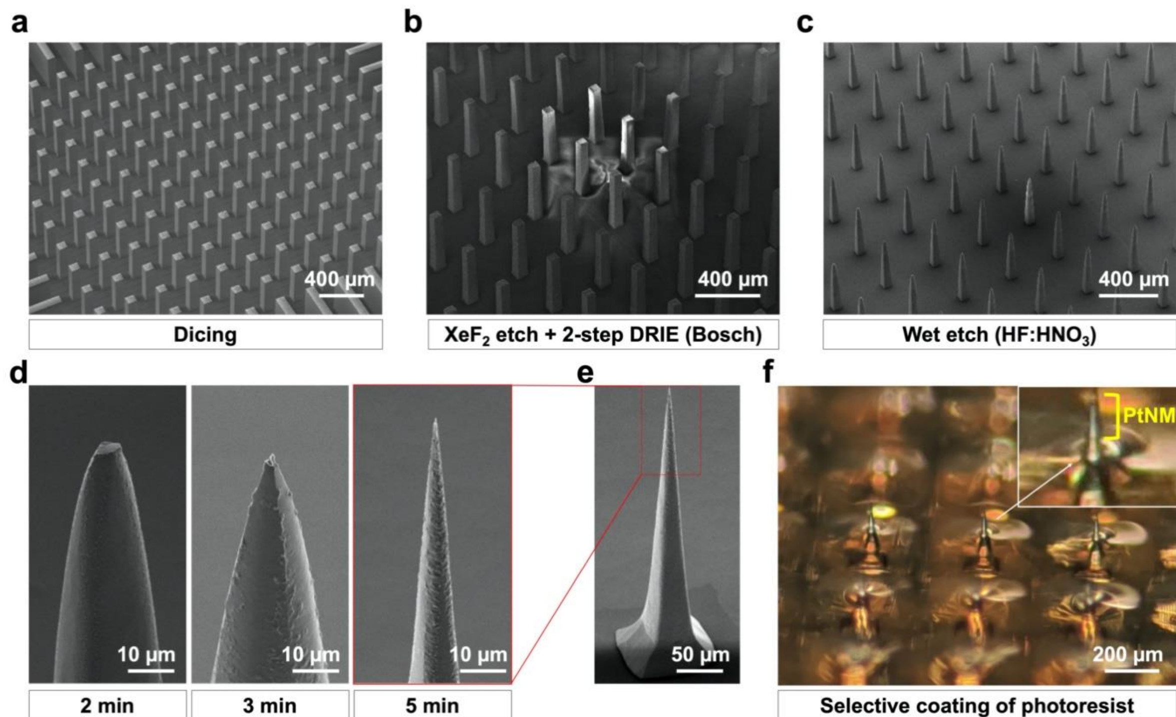


Figure S12. Optimization of sub-300 µm-tall SiMN micromachining and etch. a, SEM image (45 degree view) of a test 400 µm needle-to-needle spacing array after micromachining with dicing saw system. **b**, SEM image (45 degree view) of the array from **a** after isotropic XeF_2 etch to thin down the micropillars, and anisotropic 2-step DRIE (Bosch etch) to expose the underlying polyimide substrate, electrically isolating microneedles from each other. **c**, SEM image (45 degree view) of the array from **b** after 2 min "static etch". **d**, SEM image (45 degree view) of tip profile of a microneedle during "static etch", from left to right; 2 min, 3 min, 5 min "static etch". **e**, SEM image (45 degree view) of a single microneedle after 5 min "static etch" with height of about 210 µm. **f**, A magnified photograph of selective coating of photoresist on SiMNA on flex.

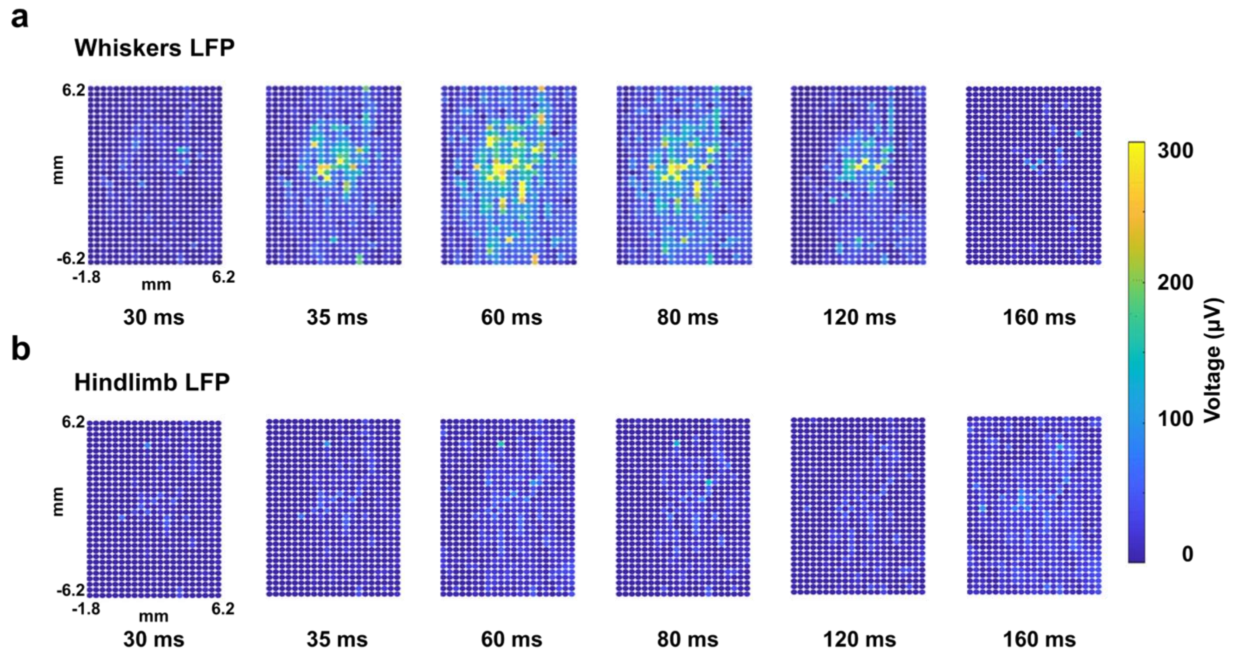
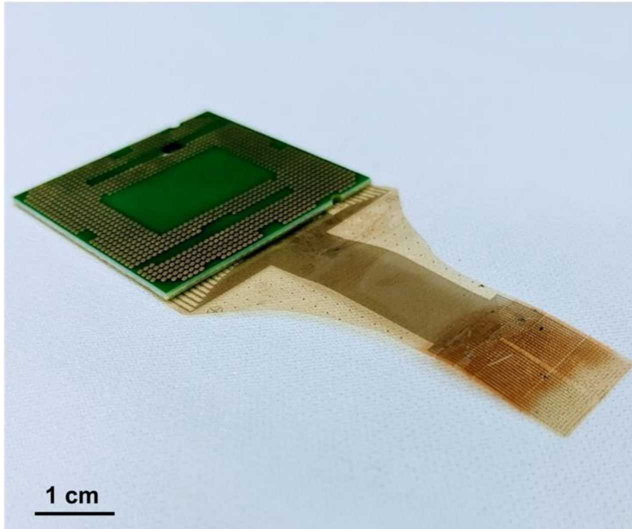


Figure S13. Validation of sensory stimulation evoked LFP responses. a, Whisker air puff stimulation evoked LFP colormaps, and **b**, hindlimb air puff stimulation evoked virtually no response as indicated in LFP colormaps from 30-160 ms post stimulus (average of 60 trials, LFP: 1-250 Hz).

a



b

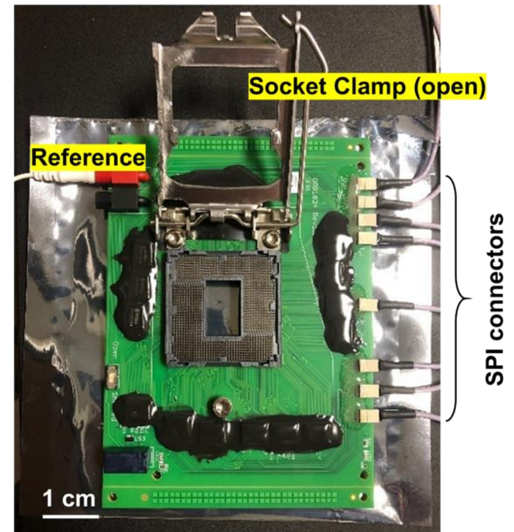


Figure S14. Custom PCB connector and ORB1024 acquisition board utilizing LGA1155 socket and Intan RHD2164 chipset. a, Example of 1024-channel device bonded to custom PCB interposer. **b**, Custom ORB1024 acquisition board with an off-the-shelf LGA1155 socket and sixteen Intan RHD2164 neurophysiological acquisition chips which condition, digitize, and stream 1024 channels in parallel at up to 30ksps/channel. The custom PCB interposer is inserted into the LGA1155 socket and the 1024-channel SiMNA on flex extends out towards left side of the image, while the differential serial peripheral interface (SPI) cables extend towards the right of the image to interface with a 1024-channel Intan Recording Controller Unit (not shown). The ORB1024 is then mounted on a stereotaxic frame before implanting the SiMNA on rat cortex.