

Supplementary Materials for

Flexible, Scalable High Channel Count Stereo-Electrode for Recording in the Human Brain

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Materials and Methods

1. Study design

Objectives of the study were to: (i) demonstrate implantable thin-film electrodes (μ SEEG) produced using reproducible, customizable, and high throughput manufacturing pipeline to be implanted in the brain such as in the operating room using similar brain implant techniques to standard clinical depth electrodes, (ii) use the electrode to record neurophysiologically relevant neural activity in multiple settings and species and; (iii) reach deep brain structures with high spatial sampling resolution and channel count with a much thinner electrode body. Following mechanical and electrochemical characterization of the properties of the new μ SEEG devices, we recorded neurophysiological activity in four species (rat, pig, rhesus macaque, and human) under general anesthesia, while awake or under monitored anesthesia care (MAC; **supplementary Table 2**). The primary goal in the physiological recordings was to use sensory (auditory or mechanosensory) or electrical stimulation as well as spontaneous neural activity to determine if the μ SEEG devices can record known neurophysiological signatures, whether in the local field potential or the single unit spiking activity (action potentials). All rodent and pig experiments were approved by the UCSD Institutional Animal Care and Use Committee (IACUC) (**supplementary Table 2**). Experimental procedures on rhesus macaques (N=2) were carried out in accordance with the Guide to the Care and Use of Laboratory Animals and approved by the IACUC at Massachusetts General Hospital (MGH; **supplementary Table 2**). We successfully gathered depth recordings of all intended stimulus-evoked activity which included baseline recordings and at least 10 trials per condition (whether injected current level or sensory stimulation). Endpoints for stopping recordings and experiments were determined by the IACUC protocols. For the NHP recordings, we planned to limit μ SEEG to 15 minutes-30 minutes for lowering and recording, particularly under anesthesia, to minimize the duration of the case.

Two human participants were recruited to participate in this study at MGH under the Partners Institutional Review Board (now the Mass General Brigham Institutional Review Board which covers MGH). The possibility of conducting research recordings were only discussed with each patient after the decision to proceed with the surgery had been made and following consultation with the treating neurosurgeon and clinical team. Further, we did not enroll patients with clearly impaired decision-making abilities. Both participants were involved voluntarily, provided informed consent, and were informed that participation in the experiment would not alter their clinical treatment in any way and that they could withdraw at any time without altering their clinical care. Finally, case selection and the decision to use the penetrating μ SEEG could be inserted into tissue which was identified by the clinical team to be resected during the course of normal clinical care. Data from each participant was de-identified prior to analysis using automatic approaches. In both cases, ongoing spontaneous activity was the control acquiring a baseline recording prior to or throughout the given recording period. Trial numbers for the second participant for the auditory stimulation task within a given subject recording were determined based on a maximum recording time of 15 minutes in the OR setting. We did not include a randomization of subject selection in this study because each recording from each individual was considered a separate dataset. Experimental conditions and a summary of all subjects are presented in **supplementary Table 2**.

Inclusion and analysis of data per experiment was decided based on the following criteria: 1) histological or physiologic confirmation that the electrode was in the targeted barrel cortex in the rodent studies; 2) if the noise level in the recording was low enough to examine neurophysiological signatures. In the former case, we had to reject 6 of 9 rodent implants as the implanted electrodes were not in the rodent barrel cortex. In the latter case of neural activity and noise levels, we were forced to exclude analyses of 2 of the 3 pig recordings due to overwhelming electrical noise. Both the tests performed with humans and non-human primates (NHPs) were included, therefore passing these criteria. Following preprocessing, trial rejection included removing trials with wayward voltage deflections >6 standard deviations from the baseline activity. Overall, only a maximum of 2 or 3 trials were removed per condition across the data sets. These data were not blinded, and we did not include a randomization of subject selection in this study. We did not use power analyses as this particular study involved observational approaches to test the functionality of the devices

2. Fabrication of electrodes

2.1. Long μ SEEG fabrication

Supplementary Fig. 1 summarizes the fabrication process of the μ SEEG electrode. Polished and cleaned photomask-grade soda lime glass plates (Nanofilm) with dimensions of 7" $\times$ 7" $\times$ 0.06" were used as substrates for the fabrication. First, a Micro-90 (International Products Corporation) diluted with deionized (DI) water (0.1%) layer was spin-coated on the glass substrate as a release layer for the polyimide electrodes in the last step of the fabrication processes. Subsequently, a sacrificial 5- μ m-thick-polyimide (PI-2611 from HD Microsystems) was initially deposited by conventional spin-coating, soft-baking, and curing (340 °C for 3 hrs, 3 °C/min in N₂ ambient) processes. This sacrificial PI layer will serve to separate the device layers, which will be constructed on top of this layer, from the glass plate. Then, a 60-nm-thick Ti hard mask for net layer formation (Ti_{net}) was formed on the sacrificial polyimide layer. The Ti_{net} was proceeded by a standard lithography, descum, metal deposition, and lift-off process using AZ5214E-IR photoresist (MicroChemicals), maskless photolithography system (Heidelberg MLA 150), UV flood exposure system (DYMAX), plasma etcher (Oxford Plasmalab 80), and e-beam evaporator (Temescal). The Ti hard mask contains via patterns with hole arrays with a diameter and a spacing of 5 μ m for both, and a 1.5 mm wide and 0.6 mm long rectangular shape for a sheath.

Then, another polyimide layer was spun-coated, soft-baked, and cured at 330 °C for 3 hrs in N₂ ambient following pre-curing of the substrate to dry out any solvent or moisture trapped in the polyimide surface under 340 °C for 3 hrs in N₂ ambient. This polyimide layer is the 1st device layer (1st PI layer) which will be role as the sacrificial bottom layer with net of holes in the final product. Then, a 60-nm-thick Ti sacrificial layer (Ti_{sacrificial}) was deposited using same method for the Ti_{net} preparation. The Ti_{sacrificial} also acts as etch-stop layer during the final net formation etching step using O₂ plasma. To increase adhesion between the 1st PI layer and another upcoming polyimide layer (2nd PI layer), hole arrays with a diameter and a spacing of 5 μ m for both are patterned along the Ti_{sacrificial} using AZ 1518 (MicroChemicals). Then, the 1st PI layer was selectively etched by O₂ plasma (50 mTorr, 200 W for 7 min), and the photoresist layer was removed by solvent cleaning processes.

After processes on the 1st PI layer, the glass substrate was baked at 330 °C for 2 hrs in N₂ ambient. Then, a 2nd PI layer was coated and cured under 320 °C for 2 hrs in N₂ ambient. Subsequently, metal traces with a width and a spacing of 3 μm for both were formed on the 2nd PI layer. Metal traces were composed of Cr/Au (10/250 nm) and the entire lithography, deposition, and lift-off process was repeated on top of the first metal lead layer to form Cr/Au/Cr/Au (10/250/10/250 nm) traces. This double-patterning process was employed to increase yield and reduce risk of photoresist particles from compromising the thin traces.

After the double-layer metal leads formation, a 30 μm-diameter-PtAg alloy was formed selectively on the individual micro-contact recording sites by photolithography, descum, and PtAg alloy co-sputtering using the maskless photolithography system with NR9-6000 (Futurrex) photoresist, plasma etcher, and DC/RF magnetron sputter (Denton Discovery 18), respectively. A 60-nm-thick Ti capping layer (Ti_{cap}) was deposited on top of PtAg alloys to prevent oxidation in air or under oxygen plasma in the following processes. The detailed fabrication methods and characteristics of PtNRs can be found elsewhere^{29, 30}. Notably, this process involves a selective etching of silver in a dealloying process, leaving behind non-toxic platinum.

A 3rd polyimide layer (3rd PI layer) was then coated and cured under 300 °C for 1 hr in N₂ ambient. On top of the 3rd PI layer, a 60-nm-thick-Ti hard mask (Ti_{outline}) was deposited and AZ5214E-IR photoresist was coated on top of the Ti_{outline} and patterned to define the outline of the electrodes. Then, SF₆/Ar plasma was used to selectively etch the Ti_{outline} through the photoresist. To accomplish a 1st deep etch step, 3 hrs of O₂ plasma was performed to etch 3rd, 2nd and 1st PI layers until exposing the Ti_{net} on the sacrificial PI layer.

Following the 1st deep etch process, a-8-μm-thick AZ12XT-20PL-10 (MicroChemicals) was spun-coated and patterned to open via holes for recording sites and printed circuit board (PCB) contact pads, and the outline of the electrodes. Then, SF₆/Ar plasma was used to etch Ti_{net} and expose the surface of the 3rd PI layer on the recording sites and the sacrificial PI layers along the outline of the electrodes. Subsequently, the exposed PI layers were etched by O₂ plasma for 1 hr.

To protect PI layers from dealloying process to form PtNRs using nitric acid, a 100-nm-Ti (Ti_{passivation}) was conformally deposited using sputtering system and a 1-μm-thick parylene C was additionally coated using a parylene deposition system (Specialty Coating Systems 2010 Labcoter). To open via holes for recording sites, a-8-μm-thick AZ12XT-20PL-10 was spin-coated and patterned again. Then, O₂ plasma and subsequent SF₆/Ar plasma was used to etch the parylene C and Ti layers including the Ti_{passivation} and Ti_{cap} to expose the PtAg alloys. The glass substrate was immersed into 60 °C nitric acid for 2 min for dealloying of PtAg alloys and rinsed in de-ionized (DI) water. The microscopic morphology of the PtNR is shown in the scanning electron microscope (SEM) images (**Fig. 1b**).

The parylene C and Ti_{passivation} were removed by O₂ plasma and 6:1 buffered oxide etchant (BOE), respectively. Then, the glass substrate was immersed in DI water to delaminate the electrodes from the glass substrate with the dissolution of the underlying Micro-90 layer.

The delaminated electrode was then flipped and transferred onto a carrier glass wafer. Following the flip transfer, the net of hole arrays was formed on the 1st PI layer by O₂ plasma removing the sacrificial PI layer and selectively etch the 1st PI layer through the

via openings in the Ti_{net} . The etching time was set longer to obtain clean opening of hole arrays. Note that the 2nd PI layer was protected, during the over etching procedure, by the $Ti_{sacrificial}$ prepared between 1st and 2nd PI layers. Lastly, the Ti_{net} and $Ti_{sacrificial}$ were all dissolved by BOE and the electrode was immersed in DI water for ~6 hrs to rinse any residual BOE.

2.2. Stylet insertion

Medical grade stainless-steel 316 stylets ($\varnothing$ 125 μ m) were used as a shuttle to guide the μ SEEG electrode to a target region. The stainless-steel was chosen as a shuttle material over a tungsten rod or a silicon shuttle because of its hydrophilic surface, which helps to avoid adhesion between the hydrophobic polyimide surfaces¹. These stainless-steel shuttles are the standard stylets used for the implantation of clinical depth electrodes. The stainless-steel stylet was mechanically polished to have smooth surface at its tip to prevent mechanical damages of the μ SEEG electrode during the stylet insertion (**supplementary Fig. 4**).

The stylet was inserted through the sheath on the stylet bottom layer and travelled along the space between the stylet upper and bottom layers where the Ti sacrificial layer was etched away. The retractable stylet provides rigidity to the polyimide electrode tip and supports implantation to the cortex without electrode deformation. The stylet insertion procedure is as follows: First, the μ SEEG electrode and the stylet was placed on a clean glass substrate (**supplementary Fig. 2a**). Then, the stylet was inserted through the stitch holes to restrict lateral movement of the stylet and the sheath of the electrode (**supplementary Fig. 2b**). To ensure that the stylet is aligned with the center of the μ SEEG, the center of the stitch hole arrays is aligned with the center of the sheath. The diameter of each hole is 800 μ m, and they are spaced 2 mm apart. **Supplementary Fig. 2c** shows a zoomed-in image of the sheath during stylet insertion. The insertion process was initiated by opening the sheath with support from a tungsten probe. Once the sheath was opened, the stylet was slid into the sheath, and the tungsten probe was gently removed. Subsequently, DI water was sprayed along the electrode strips to open the space between the stylet upper and lower layers. In a few seconds after DI water spraying, the stylet could be travelled along the space (**supplementary Fig. 2d and supplementary Fig. 2e**) and finally reached to the tip of the electrodes (**supplementary Fig. 2f**). **Supplementary Fig. 2g** shows scale of the μ SEEG electrode after the stylet insertion and comparison with the clinical electrode indicating that a length of the stylet inserted part of the μ SEEG electrode is long enough to access to the deep brain structure. A cross-section view SEM image (**supplementary Fig. 2h**) shows the stylet upper and bottom layers with the stylet in between after recordings from non-human primate (NHP). The tip of the μ SEEG electrode was deliberately opened by a razor blade to observe cross-sectional details of the μ SEEG electrode with bio-materials remained on. **Supplementary Fig. 2i** displays a cross-section SEM image of the μ SEEG electrode after the stylet retraction, showing negligible deformation and suggesting that the stylet insertion and retraction does not cause permanent deformation of the probe sheath. During the stylet insertion, the flexibility of the polyimide allows the μ SEEG electrode to adjust its shape, inflating it from two straight lines with a length of 1.24 mm to an oval shape with a longer length of 1.02 mm and a shorter length of 0.25 mm, which corresponds to the diameter of the stylet. This indicates that the stress on the polyimide

is relaxed as the electrode shape changes during stylet insertion. This is also observed under optical microscope (OM) image (**Supplementary Fig. 2e**) showing different light reflection from the edge of the electrode depending on whether the stylet has already been implanted or is yet to be implanted.

2.3. Short μ SEEG fabrication

The short 64 channel μ SEEG electrode has a large space (flaps) near the interconnection metal leads for surgeon's convenience for electrode handling. It is also designed to bond with anisotropic conductive films (ACF) with commercial off-the-shelf ribbon cables to connect the electrode to the external characterization circuitry (**supplementary Fig. 8a**). The electrode consists of three polyimide (or parylene C) layers to not only passivate metal leads, but also carry the stylet during implantation (**supplementary Fig. 8b**). Au was used to serve sacrificial layer to separate 1st and 2nd polyimide layers for the stylet insertion by dissolving of Au layer using Au etchant (TFA, Transene Company, Inc.). A metal lead layer of Cr/Pt (20/100 nm) was embedded between 2nd and 3rd polyimide layers, but recording electrodes were via-opened. To enhance recording qualities, PEDOT:PSS was electro-deposited on the Cr/Pt recording electrodes. PEDOT:PSS was electrodeposited from 0.01 M 3,4-Ethylenedioxythiophene (EDOT) in 2.0 g per 100 mL Poly(sodium 4-styrenesulfonate) aqueous dispersion under galvanostatic conditions at a potential of 0.9 V versus Ag/AgCl in a three electrode setup, i.e., Ag/AgCl electrode as a reference electrode, a large Pt electrode as a counter electrode, and the Pt contacts on the probes as the working electrodes, at a constant temperature of 27 °C using a Gamry potentiostat (Gamry Interface 1000E; Gamry Instruments). Polymerization was driven for 20 s at current density of 5 mA/cm².

After the stylet was inserted in the μ SEEG electrode, the large space and bonding pad regions were bent due to strain of the polyimide layers because of its thickness and geometry (**supplementary Fig. 8c**), but it is confirmed that a region for the recording electrodes remained straight with the stylet (**supplementary Fig. 8d**) and PEDOT:PSS electrodes, Cr/Pt metal leads, and polyimide layers are not damaged (**supplementary Figs. 8e and 8f**). **Supplementary Fig. 8e** also displays a detail of the electrode layout showing hole arrays patterned along the metal leads to help wet etching of the Au sacrificial layer. The diameter and center to center spacing of electrode sites is shown as 20 and 60 μ m, respectively (**supplementary Fig. 8f**), and the electrode consisted of 64 channels along 3.80 mm in a laminar manner. The dimension and the number of electrodes can be adjusted based on the target of merit.

Electrochemical impedance spectroscopy (EIS) of the electro-deposited PEDOT:PSS was performed, in 1X phosphate buffer saline (PBS) solution (**supplementary Fig. 8g**). Three electrode configuration i.e., PEDOT:PSS electrodes as the working electrode, Ag/AgCl electrode as a reference electrode, a large platinum electrode as a counter electrode was used. 10 mV root mean square (RMS) sinusoidal signal with zero DC bias were applied and the frequency was swept from 1 Hz to 10 kHz using a Gamry potentiostat (Gamry Interface 1000E; Gamry Instruments). Electrochemical impedance at 1 kHz is commonly used as the benchmark for the characterization of neural electrodes, as this frequency corresponds to spiking activity². The average impedance magnitude of PEDOT:PSS electrodes across 64 channels was 33.0 ± 2.47 kilohms at 1 kHz and their phase indicates their faradaic electrochemical interfaces. After stylet insertion, the

average impedance magnitude maintained similar values of 35.0 ± 3.67 kilohms on 64 channels, still lower and consistent contrast to Pt electrodes (**supplementary Fig. 8h**), indicating that the stylet was successfully inserted between the polyimide layers without any damage to the channel or PEDOT:PSS separation from Pt electrodes. **Supplementary Fig. 8i** displays averaged impedance magnitude at 1 kHz for 7 PEDOT:PSS μ SEEG electrodes, before and after stylet insertion. The channel yield before and after stylet insertion is 99.8 ± 0.5 % and 99.3 ± 0.7 %, respectively. The channel yields below reflect the negligible electrode losses during the stylet insertion.

2.4. Surface μ ECoG fabrication

We fabricated a μ ECoG electrode array to use during depth recordings to address the location of the barrel cortex before μ SEEG implantation. The μ ECoG electrode array was placed on the region where the barrel cortex is expected to be located and we performed recordings with whisker airpuff stimulation. After we confirmed that the μ ECoG electrode array captures activities from the whisker airpuff stimulation, the μ SEEG was implanted between the columns of the μ ECoG electrode array. Parylene C was chosen as the surface μ ECoG electrode material because of its superior conformability and hydrophobic surface which makes stable electrical and mechanical contact with the surface of the cortex³. The μ ECoG electrode is designed to perform dual column recordings while the μ SEEG electrode measures intracortical signals between the columns (**Supplementary Figs. 13a and b**). It is also designed to bond with ACF with commercial off-the-shelf ribbon cables to connect the device to the external characterization circuitry (**supplementary Fig. 13a**). The fabrication process of parylene C μ ECoG electrode was reported elsewhere^{4, 5}.

First, an anti-adhesion layer, Microo-90 diluted with DI water (0.1 %) was spun-coated on a 4" Si carrier wafer to enable parylene C device release from the Si carrier wafer. A 1st parylene C layer (3 μ m) was deposited on top of the anti-adhesion layer by chemical vapor deposition (Specialty Coating Systems 2010 Labcoter). A Cr/Au/Ti (10/100/50 nm) metal lead layer was evaporated and patterned via lift-off process. Cr layer serves as an adhesion layer between Au conduction layer and parylene C. Ti layer was deposited to protect Au layer during later via dry-etching process. A 2nd parylene C insulation layer (3 μ m) was deposited. A Ti (50 nm) layer was deposited and patterned via lift-off process on the second parylene C layer as an etch hard mask against O₂ plasma etching. The Ti hard mask was patterned to define 1) via openings of the Au electrode sites and bonding pads, 2) shape of the device body and 3) perfusion holes on parylene C layers. Perfusion holes can help minimizing the excessive cerebrospinal fluid (CSF) around the electrode sites⁶. The Ti hard mask and protection layer was removed by BOE and rinsed with DI water, leaving fresh Au surface at the electrode sites. The parylene C device was released from the Si carrier wafer by removing Micro-90 with DI water. The bonding pad region of the probe (Cr/Au) was bonded with ACF and commercial off-the-shelf ribbon cables and additionally encapsulated by kapton tape to prevent saline or CSF leakage (**supplementary Fig. 13c**). To enhance recording qualities, PEDOT:PSS was electrodeposited on the surface of Au electrode sites using the technique described above. The surface probe consists of two column 32 channels along 3.12 mm in a laminar manner and the diameter and pitch of electrode sites is 20 and 100 μ m, respectively (**Supplementary Figs. 13d to f**).

Electrochemical impedance at 1 kHz was measured using Intan RHD2000 USB interface board (Intan Technologies) in 1X phosphate buffer saline (PBS) solution with a stainless-steel needle as a reference electrode (**supplementary Fig. 13g**). The average impedance magnitude of Au electrodes was 739 ± 85.4 kilohms, which was decreased to 33.6 ± 3.21 kilohms after PEDOT:PSS electrodeposition on Au electrodes, with uniform distribution of the impedance across the channels (**supplementary Fig. 13h**).

3. Mechanical characterizations of μ SEEG electrodes

3.1. μ SEEG insertion benchtop tests

Insertion of the prototype μ SEEG was tested on a phantom brain model. A transparent brain phantom gelatin model was prepared by mixing and dissolving a gelatin powder (Knox; Kraft Foods, Inc.) with weight concentration of 5.3 %. Knox gelatin with this concentration was reported to provide similar shear modulus to the mouse brain⁷ and its transparency helps in visualization of the μ SEEG electrode movement in the gel. We designed and printed a custom-made 3D printed holder that can be attached to the stereotaxic micromanipulator while holding the prototype μ SEEG (**supplementary Fig. 7a**). The prototype μ SEEG, held by the 3D print holder, was slowly inserted into the gelatin using the z-axis control handle of the stereotaxic micromanipulator. After the prototype μ SEEG was inserted, the stainless-steel stylet was manually grasped by a tweezer and extracted from the prototype μ SEEG. The implantation of the prototype μ SEEG and displacement after stylet extraction were recorded using a camera with a macro lens (**supplementary Fig. 7b**). The prototype μ SEEG was implanted without any deformation with help of the stylet. Also, the displacement of the prototype μ SEEG before and after stylet extraction was less than 10 μ m.

3.2 Pull measurement of μ SEEG

We investigated tensile stress of μ SEEG using pull measurement to examine the mechanical stability of our electrode and compare it with clinical depth electrode (PMT electrode). The μ SEEG was integrated on the pressure sensor system (voice coil-powered linear actuator system with internally integrated force sensor; V-275 PIMag Voice Coil Linear Actuator). A customized 3D printed sample mount was attached onto the actuator (**supplementary Fig. 5a**) and a tip of the μ SEEG was placed on the sample mount followed by strong fixation using epoxy. Rest of the electrode was attached on a main body of the system and fixed by epoxy as well (**supplementary Fig. 5b**). We applied force to the actuator to exam tensile strength upon the electrodes break (indicated by red arrow) and obtained that the critical tensile strength. The critical strength of parylene C μ SEEG and polyimide μ SEEG are 0.6MPa (24 mN with an area of the stress of $4e^{-8} m^2$) and 1 – 2.5 MPa (16 – 37 mN with an area of the stress of $1.5e^{-8} m^2$), respectively. Meanwhile, the PMT electrode that was anchored on two polyurethane tube regions around a Pt contact was damaged when 14 kPa (48 mN with an area of the stress of $3.5e^{-7} m^2$) was applied to the PMT electrode (**supplementary Fig. 5c**). The illustration of the area of the stress of electrodes is illustrated in **supplementary Fig. 5d**.

Mechanical stress introduced by stylet insertion was investigated using finite element method (FEM) analysis (**supplementary Fig. 9a and b**). The analysis indicates that the maximum strength applied to the films during the stylet insertion (0.11 MPa) are lower than the critical strength of the polyimide (1 – 2.5 MPa) and parylene C (0.6 MPa), as shown in **supplementary Fig. 9a**. Deformation of the films are lower than 4 μ m, which

can be considered as negligible from the scale of the electrodes, as shown in **supplementary Fig. 9b**. In theory, according to the simulation, the electrode should not sustain damage during stylet insertion. However, in practical scenarios, there are additional variables that can introduce stress to the electrode, such as misalignment of the electrodes in DI water (twisted or curved) and the angle at which the stylet is inserted, which may not be perfectly horizontal to the surface. While the polyimide layers are resilient and can withstand external stress arising from real-world conditions (**supplementary Fig 9c – e**), the parylene C layers appear to be approaching their critical limits (**supplementary Fig 9f – h**).

3.3 Endurance of μ SEEG

The reliability of μ SEEG was evaluated through an accelerated aging test to validate the use of our electrode in semi-chronic applications. The electrode was immersed in 50 °C saline solutions for over 74 days to assess its yield after >150 days of implantation⁸. OM images of the μ SEEG before and after the aging test showed negligible changes (**Supplementary Fig 6a and 6b**), indicating good structural integrity. During the test, impedance measurements at 1 kHz exhibited minor fluctuations in both mean and standard deviation values (**Supplementary Fig 6c**). Prior to the accelerated aging test, we measured 49 functional contacts with an average impedance of 94 ± 26 k Ω . After the test, we observed 52 functional contacts with an average impedance of 96 ± 28 k Ω (**Supplementary Fig 6d**). These results demonstrate the robustness of the μ SEEG electrode even under accelerated aging conditions. In addition, we performed 84,000 cycles of lead bend testing, exceeding 90° bends as per EN 45502 standards, to validate the capability of our electrode for general application in active implantable medical devices⁵. A robotic gripper model 2F-140 (Robotiq) was used for the bending tests. After bending, 50 contacts (average impedance of 93 ± 27 k Ω) were functional. A few changes in number of functional channels are attributed to different contact latching between the PCB and the custom ironwood electronics socket that requires latching to form robust electrical connections between PCBs and pins in the socket on the acquisition board and is minimal indicating resilience of our electrodes to bending cycles.

3.4 Mechanical stability of PtNR

PtNR μ SEEG electrodes (N = 3) after extraction from the NHP brain was investigated by SEM (**supplementary Fig. 11**). It was observed that PtNRs remained intact underneath the adsorbed tissue that are left on the surface of the electrodes (**supplementary Fig. 11a**). A higher magnification SEM image (**supplementary Fig. 11b**) shows PtNRs morphology similar to that of before implant (**Fig. 1b**).

4. Rat recording

4.1. Acute rat recordings: surgical procedures and sensory stimulation

Acute *in vivo* electrophysiological recordings were performed on the rat primary somatosensory “barrel” cortex (S1) with the μ ECoG electrode and the μ SEEG electrode. The rat was anesthetized and craniotomy was performed under isoflurane anesthesia. The body temperature of the rat was maintained at 37 °C with a heating pad. Craniotomy

and dura removal were performed over the right barrel and surrounding cortical region. Following electrode placement, the rat was transitioned to ketamine/xylazine anesthesia for recording.

Tactile stimulation was performed by delivering air puffs to the whisker pad. Air puffs were pressure-injected through a glass micropipette using a PV830 pneumatic picopump (World Precision Instruments, Inc.) with 1 s pulses ($n > 10$ trials per location). The contralateral (left) whiskers with respect to the recording sites were deflected by air puff (± 2 mm). First, the whole contralateral whiskers (multi-whisker) were stimulated. Then single whiskers (C1-3, D1-3, E1-4) were stimulated by placing the pipette as close as possible to each whisker to avoid deflection of the neighboring whiskers. Recording data were collected for 60 s for each whisker.

4.2. Acute rat recordings: histology of the rat brain

Prior to the insertion to the cortex, the back of the tip of the μ SEEG electrode was painted with a fluorescent dye, Dil (1,1'-Diocetadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, 0.1% in ethanol; Invitrogen) to visualize the μ SEEG electrode track in histopathological sections and verify the placement of the μ SEEG electrode with respect to cortical depth. Following the electrophysiological recordings, rats were sacrificed and perfused with 4% paraformaldehyde (PFA), and the brains were fixed for 2 hrs in 4% PFA. Brains were transferred to a 30% sucrose solution, and then embedded in OCT matrix and stored at -80. Slices were sectioned on a cryostat at 50 μ m. Free-floating sections were washed in phosphate buffered saline (PBS) + 0.1% Triton for 10 min, and then incubated in 1:50 Neurotrace 500/525 (ThermoFisher) for 30 min. Sections were washed in PBS + 0.1% Triton for 10 min, followed by PBS. Sections were mounted on slides and coverslipped using ProLong Gold (ThermoFisher). Slices were imaged using a Keyence BZX-700 at the UCSD Department of Neuroscience Microscopy Core (supported by NS047101). From the histology result (**supplementary Fig. 14**), the tip of the acute μ SEEG electrode was estimated to be 1750 μ m deep in the cortex, reaching layer VI.

4.3. Chronic rat recordings: implantation of μ SEEG electrode, surgical procedures, and sensory stimulation

The short 32 channel μ SEEG electrode for chronic rat recordings consists of 32 channels of microcontacts that are spread along the 2-mm-scale μ SEEG electrode, which are connected to the metal pads for interfacing with PCB (**supplementary Fig. 22a**). The metal pads and PCB are bonded by Ag epoxy. The back side of the PCB has electrical and mechanical contact with zero-insertion-force (ZIF) connector. The ZIF connector was activated with a lever to close the contact after flat flexible cable (FFC) insertion into the ZIF connector to make secure electrical and mechanical contact.

For implantation of the chronic μ SEEG electrode, first, a C-shape mount was fixed onto the skull near the craniotomy using screws and it was then bonded with the headstage using a super glue. Then, the chronic μ SEEG electrode with PCB and ZIF with FFC inserted was temporarily attached on the glass slide and the glass slide was connected to the micromanipulator (**supplementary Fig. 22b**). During the implantation, position of the chronic μ SEEG electrode was precisely controlled by micromanipulator. After the implantation, FFC was unplugged from the ZIF and the chronic μ SEEG electrode with

PCB and ZIF are securely protected by the headstage and fixed onto the skull due to the screw-fixed mount. The craniotomy was sealed by ultraviolet curable medical glue (Tetric EvoFlow). Stimuli to induce neural activity included whisker, paw, and trunk mechanical stimulation including air puffs delivered >10 times/trials and the recording data from each trial were averaged.

During the acute experiment, implanting the μ SEEG electrode in the rat barrel cortex was relatively straightforward as we had the flexibility to create a larger craniotomy and position the μ ECoG arrays to locate the barrel cortex before electrode implantation. However, for chronic implantations, additional preparations are necessary. Firstly, the headstage must be securely fixed to the rat's skull prior to the implantation. Secondly, it is crucial to keep the size of the craniotomy as small as possible to minimize the risk of infections. These requirements increase the complexity of implanting the μ SEEG electrode into the rat barrel cortex and resulted in successful detection of strong whisker stimulation-evoked activities in only 3 out of 9 rats.

4.4. Chronic rat recordings: histology of the rat brain

At the termination of the chronic recordings (by four weeks after implant), brains were removed and briefly (2 hrs) fixed in 4% PFA. The cortices were removed and flattened between glass slides in PBS, and then the flattened cortex further fixed in 4% PFA overnight, and then allowed to sink in 30% sucrose. For measuring implantation scar, rats were implanted with a dummy clinical electrode or μ SEEG and allowed to recover for two weeks ($n = 1$ / electrode). Animals were perfused with 4% PFA and then sunk in 30% sucrose. Whole brains or flattened cortices were sectioned on a cryostat in the horizontal plane from at 40 μ m. Sections were washed with PBS and stained overnight with one or more of the following: chicken anti-NeuN (1:500, Sigma ABN91), Cy3-conjugated mouse anti-GFAP (1:1000, Sigma C9205), rabbit anti-VGLUT2 (1:500, Abcam ab216463). After wash, slices were incubated in secondaries of goat anti-chicken 488 (Sigma SAB4600039), goat anti-chicken 647 (Sigma SAB2600184), or goat anti-rabbit 488 (ThermoFisher A-11008) at 1:300 for 1 hour, rinsed, and mounted with ProLong with DAPI. Slides were imaged on a Nikon Eclipse Ti2-E with a DS-Q12 CMOS camera at the UCSD Nikon Imaging Core Images were analyzed using ImageJ (**supplementary Figs. 10a to d**). In our observations from four chronic (2 weeks) placement, we observed no significant difference in the number of Neun-positive cells surrounding the lesion between the μ SEEG and the clinical grid, but there was a small non-significant improvement with the μ SEEG electrode (2-way ANOVA, $F(1, 72) = 3.290$, $P = 0.0739$) (**supplementary Figs. 10e and 10f**).

4.5. Rat recording methods

We successfully gathered depth recordings of all intended stimulus-evoked responses and gathered tissue for histological evaluation. These data were not blinded, and we did not include a randomization of subject selection in this study. The data from the μ SEEG electrode was acquired at 15 – 20 kHz and filtered by default Intan settings with cutoffs of 1 Hz to 7.5 kHz. The impedance tests of the electrodes during the experiments and recordings in the animals carried out using the Intan RHD2000 software from Intan Technologies (Los Angeles, CA). Data was extracted and processed using MATLAB (Mathworks, Natick, MA).

5. Pig recording

5.1. Pig surgical details and spinal cord stimulation

The pig experiments were approved by the University of California San Diego (UCSD) Institutional Animal Care and Use Committee. Yucatan pigs were induced with isoflourance and intubated. Once anesthetized, the animal was mounted into a stereotaxic frame in the prone position. Vital signs were monitored including heart rate, blood pressure, EtCO₂, respiratory rate, and blood oxygenation. The cranial surgical site was centered over the motor cortex of the frontal lobe and somatosensory cortex of the parietal lobe. After immobilization, a skin incision (2-4 inches long) was made and unilateral craniotomy was performed using a high speed surgical drill. The bone was removed to create a window approximately 25 mm x 15 mm. The underlying dura was incised and reflected medially towards the sagittal sinus. The cortex was hydrated with normal saline solution. A flexible sterile multielectrode implant was then placed on the surface of the exposed cortex. After placement of the electrode into the cortical surface, the electrode ribbon (<1 cm in width) was connected to an Intan recording controller. For spinal exposure, a midline incision and laminectomy were performed with a Kerrison rongeur to sufficiently expose the target thoracolumbar enlargement of the spinal cord. Following laminectomy, a longitudinal incision was made in the dura and arachnoid tissue was dissected to allow for exposure of the spinal cord. Spinal cord stimulation was performed with a handheld Ojemann stimulator with two ball tips (Radionics Inc., Burlington, MA) with 0.5 cm spacing between electrode tips. Isoflurane anesthesia was halted following completion of surgical procedures and was replaced with IV Propofol for the duration of the stimulation testing.

5.2. Pig recording methods

We successfully gathered depth recordings of all intended stimulus-evoked responses. These data were not blinded, and we did not include a randomization of subject selection in this study. The data from the μ SEEG electrode was acquired at 15 – 20 kHz and filtered by default Intan settings with cutoffs of 1 Hz to 7.5 kHz. The impedance tests of the electrodes during the experiments and recordings in the animals carried out using the Intan RHD2000 software from Intan Technologies (Los Angeles, CA). Data was extracted and processed using MATLAB (Mathworks, Natick, MA).

6. NHP recording

Experimental procedures on rhesus macaques were carried out in accordance with the Guide to the Care and Use of Laboratory Animals. All efforts were made to minimize discomfort, and the Institutional Animal Care and Use Committee at the Massachusetts General Hospital monitored care and approved all procedures. We tested μ SEEG electrodes in two settings: 1) recording from a short μ SEEG in an anesthetized NHP the operating room for recording visual cortical dynamics and 2) recording from a long μ SEEG in an awake NHP with the electrode lowered through an implanted chamber with a standard Microdrive (**supplementary Table 2**).

An intraoperative, intracranial neurophysiology recording were acquired from one adult male rhesus macaque (*Macaca mulatta*, age 11). The macaque was placed under

general endotracheal anesthesia (isoflurane) and placed into a stereotactic frame (Kopf; Kujunga, CA). A craniotomy over the visual cortex was performed using standard anatomic landmarks, and cortex was carefully exposed and the short μ SEEG electrode was implanted. Signals were recorded using a custom Intan Recording System as described previously^{4,9}.

In the awake NHP preparation, one adult male rhesus macaque (*Macaca mulatta*, age 14) was implanted with two recording chambers that allowed access parietal lobe, temporal lobe, and thalamic brain regions. A fitted Microdrive was placed on the top of the chamber to allow insertion through the dura. Following recording, trajectories were calculated by mapping the depths calculated from the Microdrive to a preoperative magnetic resonance imaging (MRI) and a postoperative CT of the chamber locations using MANGO (San Antonio, TX). To visualize the locations in 3D, we used the Scalable Brain Atlas with the Calabrese atlas (<https://scalablebrainatlas.incf.org/>;^{10,11} exported into Blender (<https://www.blender.org/>). The electrode was lowered acutely to three different depths to record neural activity. No clear adverse behavioral effects were observed before or after electrode implant or explant. Recordings of the long μ SEEG involved the use of the 1024 Intan Recording system with a 1024 channel Intan chip clamped device developed for recording thin film microelectrodes⁵.

For both preparations, data from the μ SEEG electrode was acquired at 30 kHz and filtered by default Intan settings with cutoffs of 1 Hz to 7.5 kHz. The majority of the data was acquired using OpenEphys acquisition graphic-user interface software (¹²; <http://www.open-ephys.org/>), with the impedance tests of the electrodes during the experiments carried out using the Intan RHD2000 software from Intan Technologies (Los Angeles, CA). Data was extracted and processed using MATLAB (Mathworks, Natick, MA). Data recorded from the μ SEEG electrode was down-sampled to 1000 Hz.

7. Human recording

7.1. Recordings in human cortex the operating room

Intraoperative recordings were performed in two participants who underwent a neurosurgical procedure at Massachusetts General Hospital (MGH). This study was approved by the Partners Institutional Review Board (now the Mass General Brigham Institutional Review Board which covers MGH). Both participants were involved voluntarily, provided informed consent, and were informed that participation in the experiment would not alter their clinical treatment in any way and that they could withdraw at any time without altering their clinical care. The patients were approached following already being scheduled to remove a portion of the left anterior temporal lobe for the treatment of epilepsy or tumor removal. The possibility of conducting research recordings were only discussed with each patient after the decision to proceed with the surgery had been made. Neither patient was medically unstable or required emergency or urgent surgery. All decisions to proceed were made following consultation with the treating neurosurgeon and clinical team. Further, we did not enroll patients with clearly impaired decision-making abilities (as determined by the primary clinical team or physician caring for the patient). The time allowed for dedicated research recording was limited per subject to minimize risk. We tested the short μ SEEG electrodes, which were inserted into tissue the clinical team identified would be resected following recording. One participant (HS1) was under general anesthesia but underwent clinically indicated stimulation of the cortical

surface prior to resection (**Supplementary Video 1**). The second participant (HS2) underwent awake cognitive testing under monitored anesthesia care (MAC) with electrocorticography (intracranial EEG) during surgery.

Neural signals were recorded using a custom Intan Recording System as described previously^{9, 13, 14}. Data from the μ SEEG electrodes were acquired at 30 kHz and filtered by default Intan settings with cutoffs of 1 Hz to 7.5 kHz using the OpenEphys acquisition graphic-user interface software (¹² <http://www.open-ephys.org/>), with the impedance tests using the Intan RHD2000 software from Intan Technologies (Los Angeles, CA).

7.2 Auditory Task in the Operating Room

The Auditory M-sequence block involves 100 trials where each trial involves a red fixation cross on a grey background while one of either two tones are played in the background. The choice of tone, whether 250 Hz or 500 Hz, per trial was randomly sampled from sequences of black or white designations pulled from an m-sequence distribution^{12, 15, 16}. These frequencies and tones have been used before to demonstrate auditory specificity intracranially in humans¹⁷.

8. Neural Data Analysis

Data was extracted and processed using MATLAB R2020a (Mathworks, Natick, MA). Data recorded from the μ SEEG electrode was down-sampled to 1000 Hz. To remove noise, the data were re-referenced by subtracting the median signal of all the channels. Local field potentials (LFPs), multi-unit activity power (300-2000 Hz) high gamma activity (HGA; 65-200 Hz) evoked by tactile stimuli were investigated. Power spectral analyses to examine the dynamics in the different conditions were performed by taking the real value of the Morlet wavelet coefficient (power) at a 1-Hz spectral resolution using a moving window of 0.5 sec moving every 10 ms, calculated using the Fieldtrip toolbox (www.ru.nl/fcdonders/fieldtrip,¹⁸). In the case of direct electrical stimulation, stimulation artifact was removed using a Tukey-windowed median filter in the 20 ms around the onset of the pulse for the single pulses¹⁹. Other measures of multi-unit activity involved measuring high gamma power (70-190 Hz) by taking the absolute value of a Hilbert transform following band pass filtering.

Current source density (CSD) provides information on the distribution of synaptic inputs for the generators²⁰. Here, CSD was calculated from LFP using the δ -source inverse CSD (iCSD) method, refined by Petterson et al.²¹:

$$(1) C = F^{-1}\Phi$$

where C and Φ represents matrix for CSD and LFP, respectively. The transformation matrix F is described by

$$(2) F_{ji} = \frac{h^2}{2\sigma} (\sqrt{(j-i)^2 + (R/h)^2} - |j-i|)$$

where h is the spacing between adjacent electrode, σ is the conductivity of the cortex and R is the radius of infinitely thin current-source discs. Here we set the σ and R as 0.3 S/m and 0.25 mm, respectively. Since CSD is calculated from LFP data of adjacent electrodes, each column was separated for calculation of CSD and the column that included a greater number of low impedance (<500 k Ω) channels (30 out of 32) was selected for the CSD analysis.

We detected bursts and calculated the burst suppression ratio (BSR) using an automated method ²² (https://github.com/drasros/bs_detector_icueeg). On the per-channel basis, this method labels each time sample as either burst or suppression. Briefly, the method uses the previous data with each channel and applies the following equations:

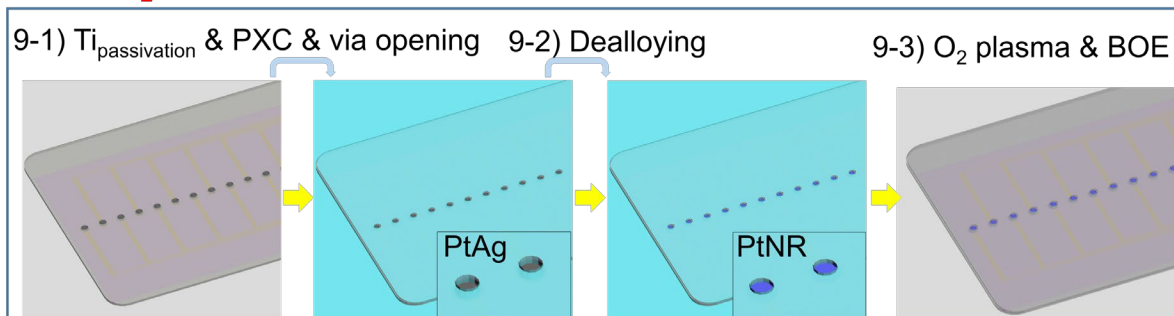
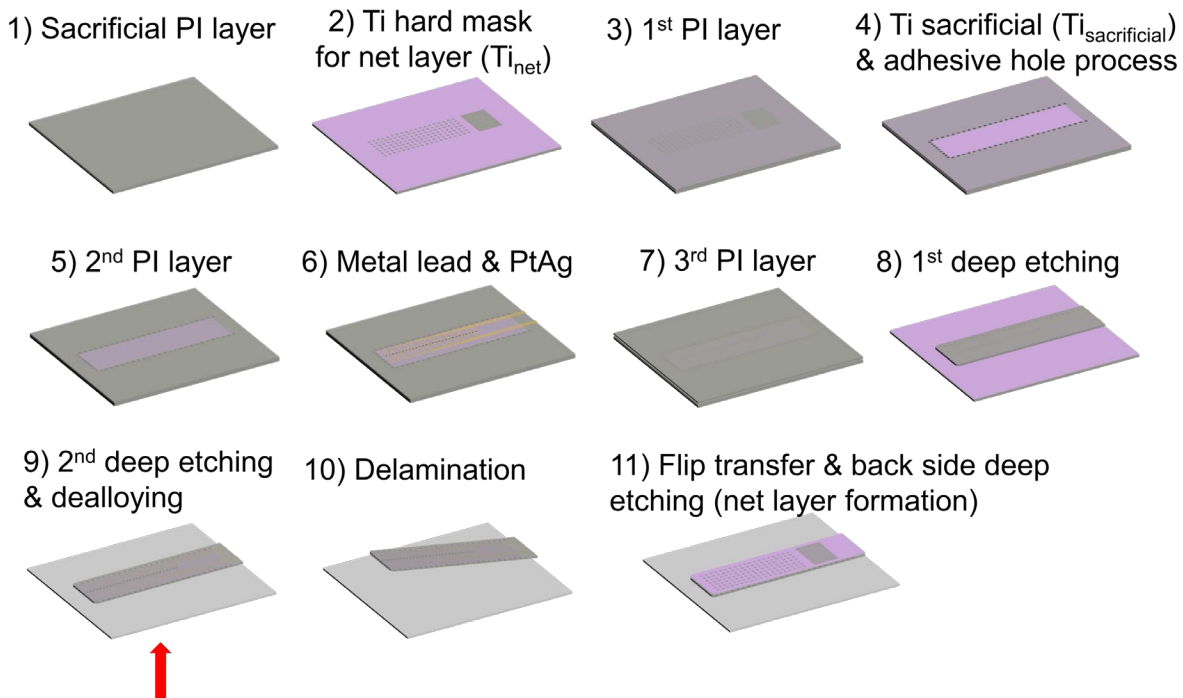
$$\begin{aligned} (3) \mu_t &= \beta \mu_{t-1} + (1-\beta) x_t \\ (4) \sigma_t^2 &= \beta \sigma_{t-1}^2 + (1-\beta) (x_t - \mu_t)^2 \\ (5) z_t &= \delta[\sigma_t^2 < \theta] \end{aligned}$$

Where x_t is the value of the normalized signal of one channel at time t , μ_t and σ_t^2 are current values of the recursively estimated local mean and variance, respectively. Finally, z_t is an indicator function that labels each data point as either a burst (0) or suppression (1). The value of β determines the balance between the effect of recent and past data set based on previously trained data ²². The classification threshold θ (i.e., the value above which a data point should be classified as burst) was adjusted to evaluate our dataset visually with values of $\theta = 100, 2000, 4000$. The value of $\theta = 2000$ was selected to reliably identify burst and suppression induced by general anesthesia. The burst suppression ratio for each recording in an anesthetized patient ($N = 1$) and NHP ($N = 1$) was evaluated as the proportion of suppression-labeled samples in a moving window (1 s duration, no overlap).

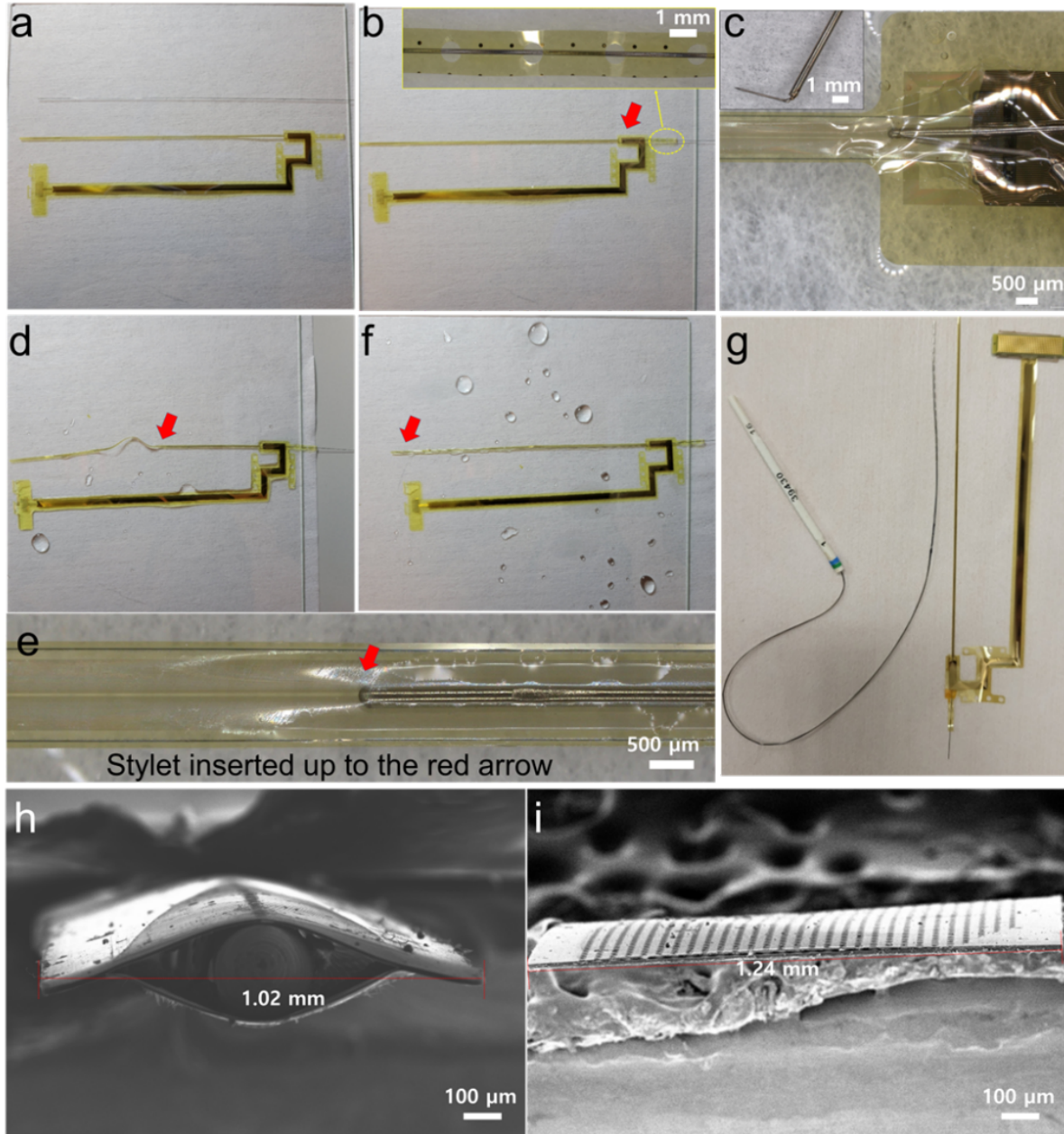
Spike sorting was performed on the long μ SEEG recordings from an NHP using Kilosort 2.5 ²³; (<https://github.com/MouseLand/Kilosort>) with further determination of single units versus multi-unit activity performed in post processing using Phy (<https://github.com/cortex-lab/phy>) and then manually curated using in-house MATLAB code to visually inspect the template as well as the waveforms assigned to each cluster. The Kilosort 2.5 parameters included: Nblocks = 0 – as no additional registration was needed according to spiking activity after the manual registration; Threshold [10, 11]. Clusters were separated into single units and MUA. Clusters of waveforms were classified as MUA if there was a mixture of distinct waveforms (examined in Phy) as well as a complicated (and abnormal) autocorrelogram. For all clusters, we measured the spike duration, peak-trough ratio, and amplitude measures (**Fig. 4**; code adapted from ²⁴; https://github.com/jiaxx/waveform_classification).

9. Statistical Analysis

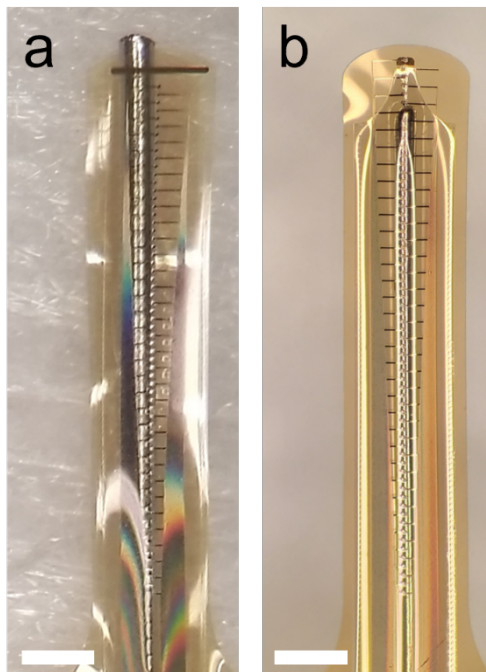
All statistical comparisons were performed using non-parametric measures, so we did not test for normality. We tested comparisons with the Kruskal–Wallis test for non-equivalence of multiple medians followed by the use of the *post hoc* Tukey–Kramer method to determine statistically separable groups. We used the Wilcoxon rank-sum test (two-sided) for comparisons between individual medians. We tested if values were significantly different from zero using the Wilcoxon signed rank test. We corrected by adjusting the target p-value (0.05) with a Bonferroni correction for the number of comparisons being done.



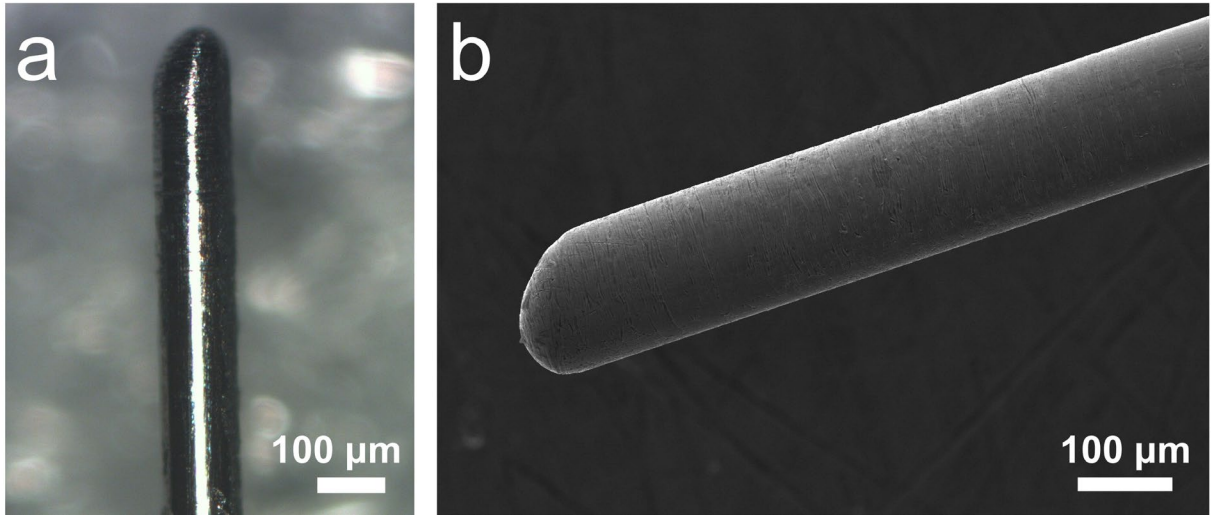
Supplementary Fig. 1. Fabrication process of μ SEEG electrode.



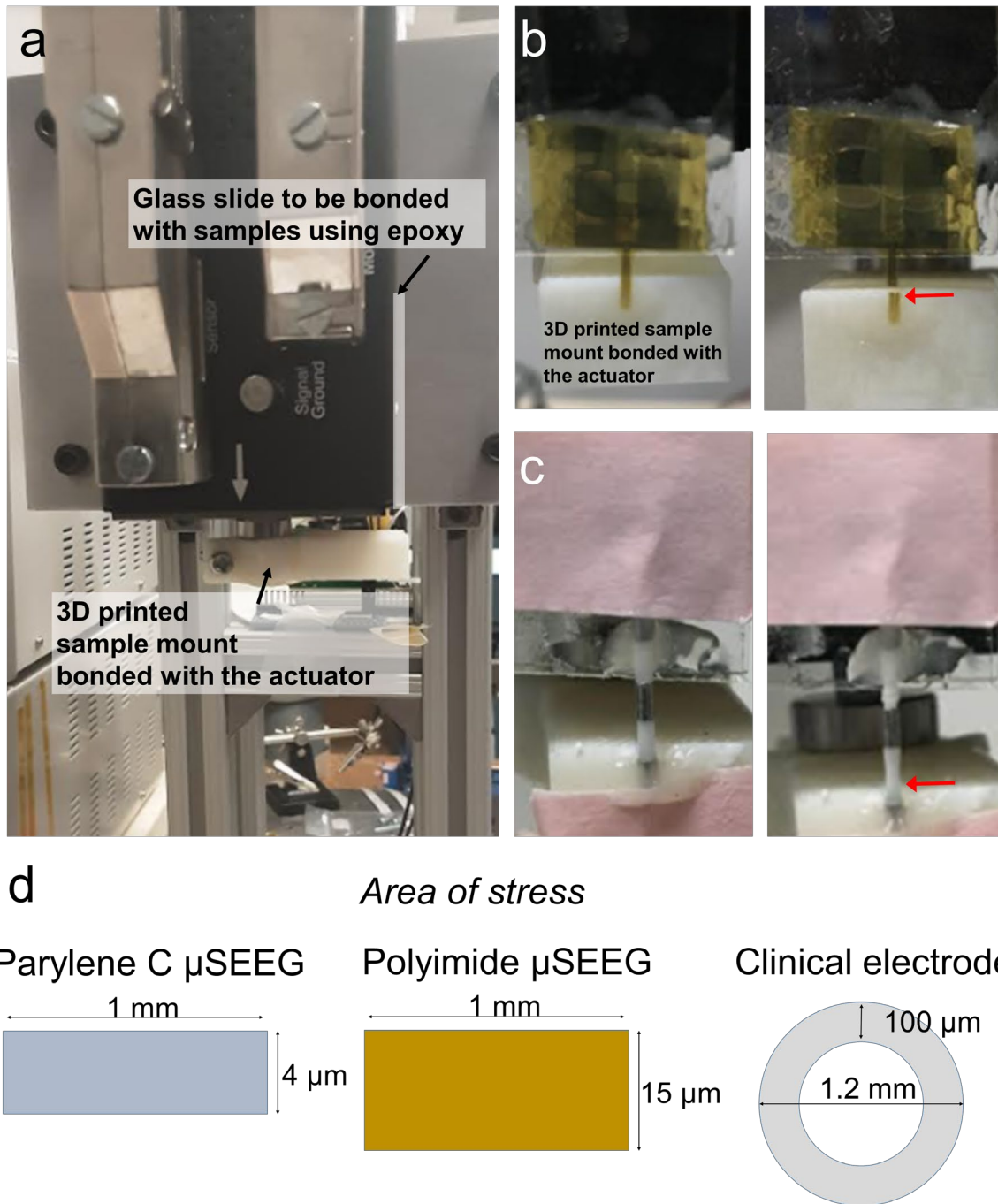
Supplementary Fig. 2. Stylet insertion into the μ SEEG electrode. (a) Before insertion. (b) Stylet aligned to the sheath. Inset shows stitch holes with the stylet interweaved. (c) Zoom-in image of the sheath and the stylet inserted with support from tungsten probe for opening the sheath. (d) During insertion. (e) Zoomed-in image of the μ SEEG during stylet insertion. (f) Stylet insertion all the way to the tip. (g) Scale comparison with conventional SEEG. Cross-sectional SEM image of the (h) μ SEEG and (i) μ SEEG after stylet retraction. The tip of the μ SEEG electrode was deliberately opened by a razor blade to observe cross-sectional details of the μ SEEG electrode with bio-materials remained on. In (i), the μ SEEG deflated after stylet retraction. The dotted surface corresponds to the net layer on the back of the μ SEEG.



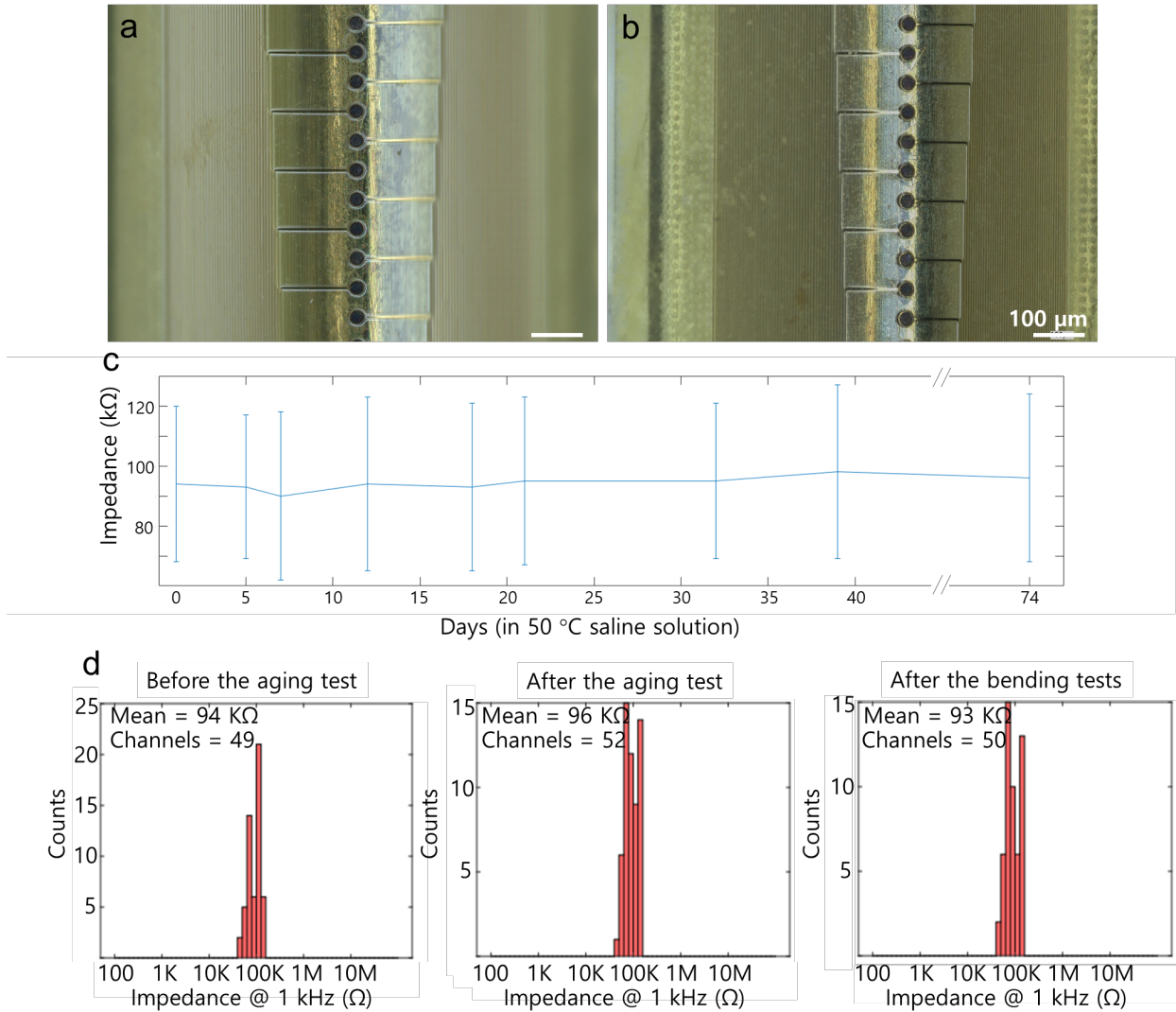
Supplementary Fig. 3. OM image of μ SEEG electrode tip after stylet insertion. (a) Without interlocking hole arrays and (b) with interlocking hole arrays. Without interlocking hole arrays, the PI layers were separated and the stylet pierced the tip. Scale bars are 500 μ m.



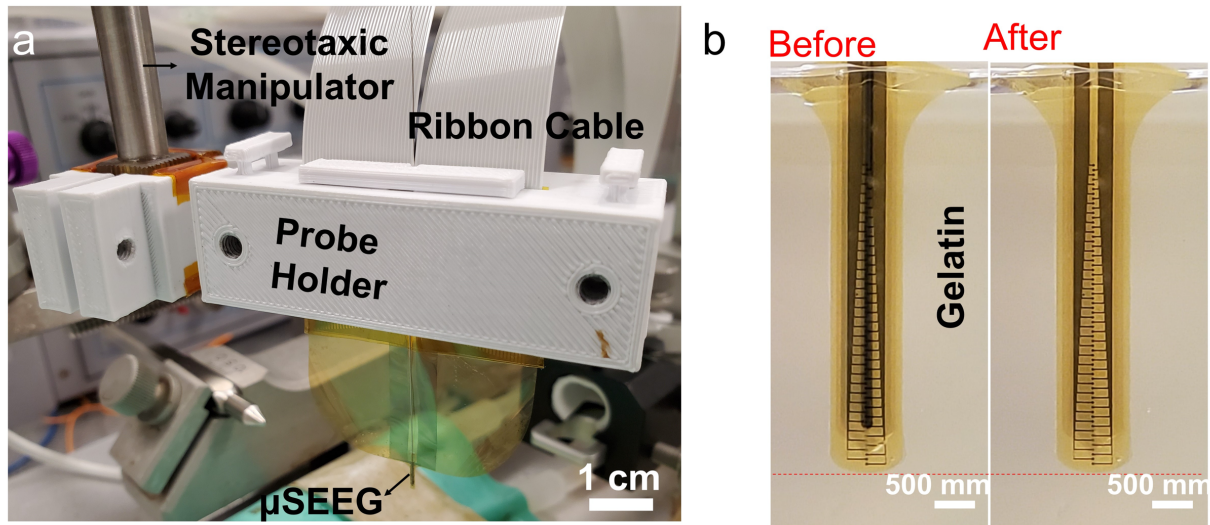
Supplementary Fig. 4. Medical grade stainless-steel stylet tip. (A) OM image and (B) SEM image of a polished stainless-steel stylet.



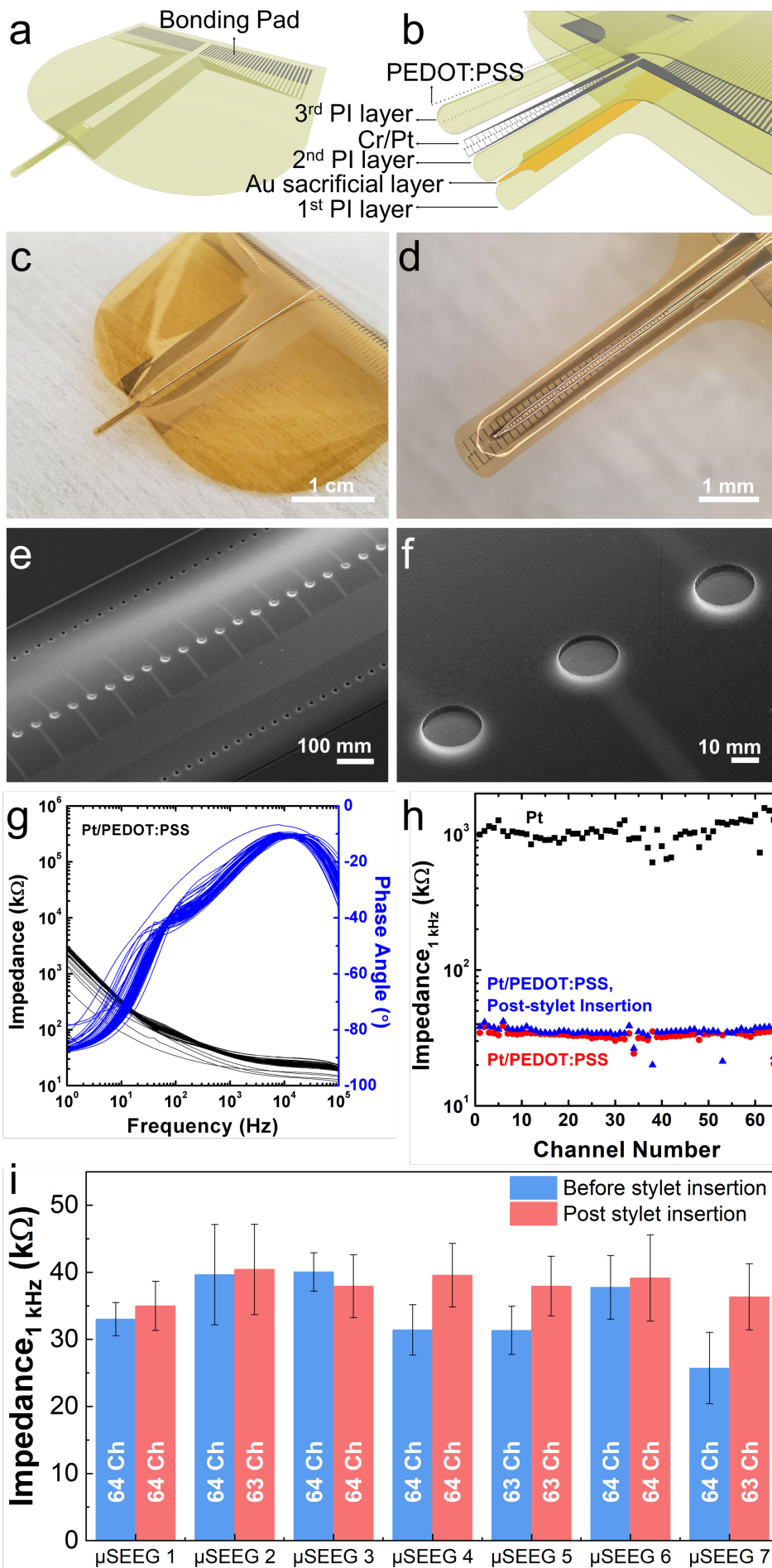
Supplementary Fig. 5. Pull measurement setup and result. (a) Measurement setup. (b) Our μ SEEG and (c) 1.2 mm diameter PMT depth electrode integrated on the measurement setup. (d) illustrations of area of stress (cross-sectional illustrations of the tested electrodes)



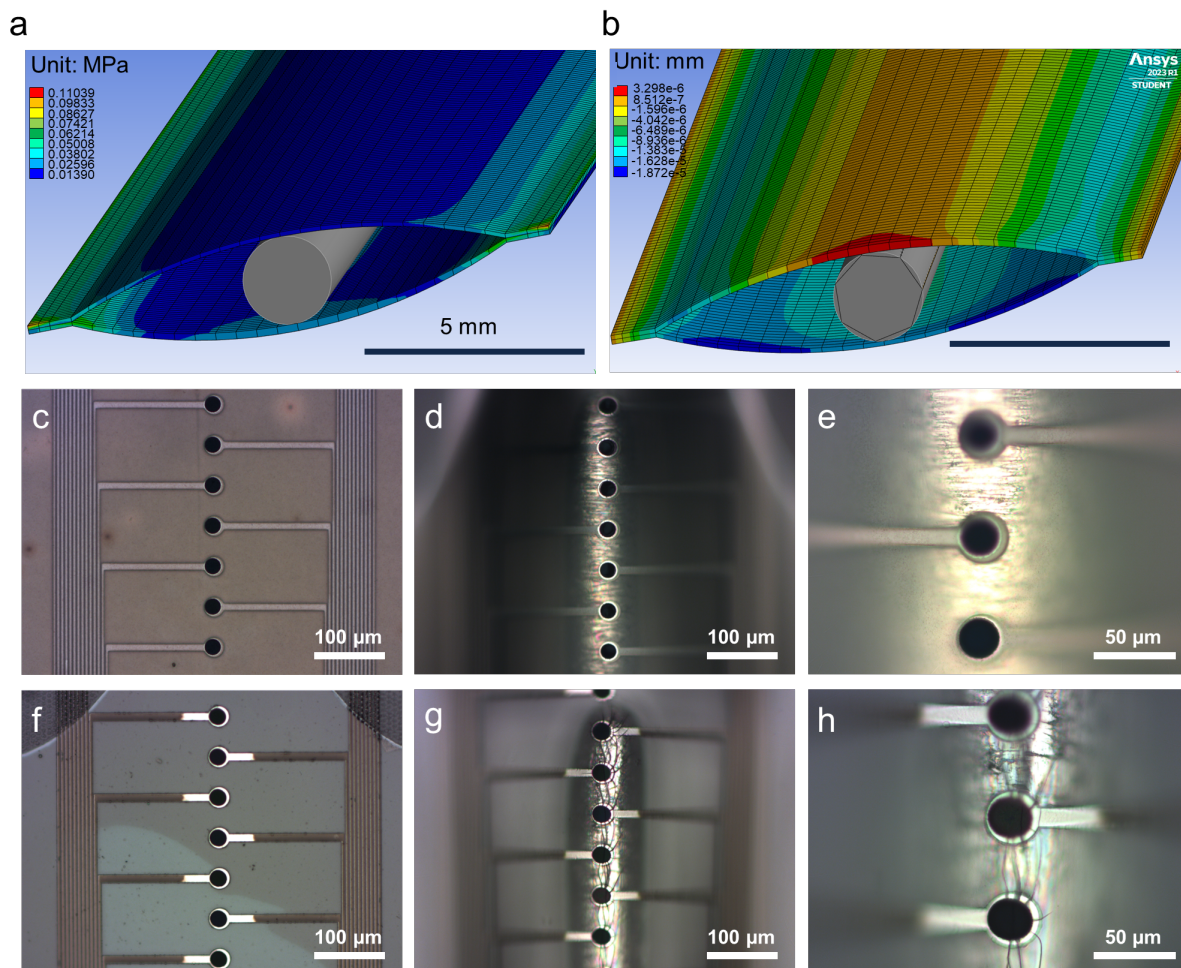
Supplementary Fig. 6. Reliability of our μ SEEG for long term implantation. OM images of our μ SEEG (a) before and (b) after the accelerated aging test. (c) Impedance of our μ SEEG at 1 kHz during the accelerated aging test. (d) Impedance histogram of our μ SEEG before, after the aging test, and after the bending tests.



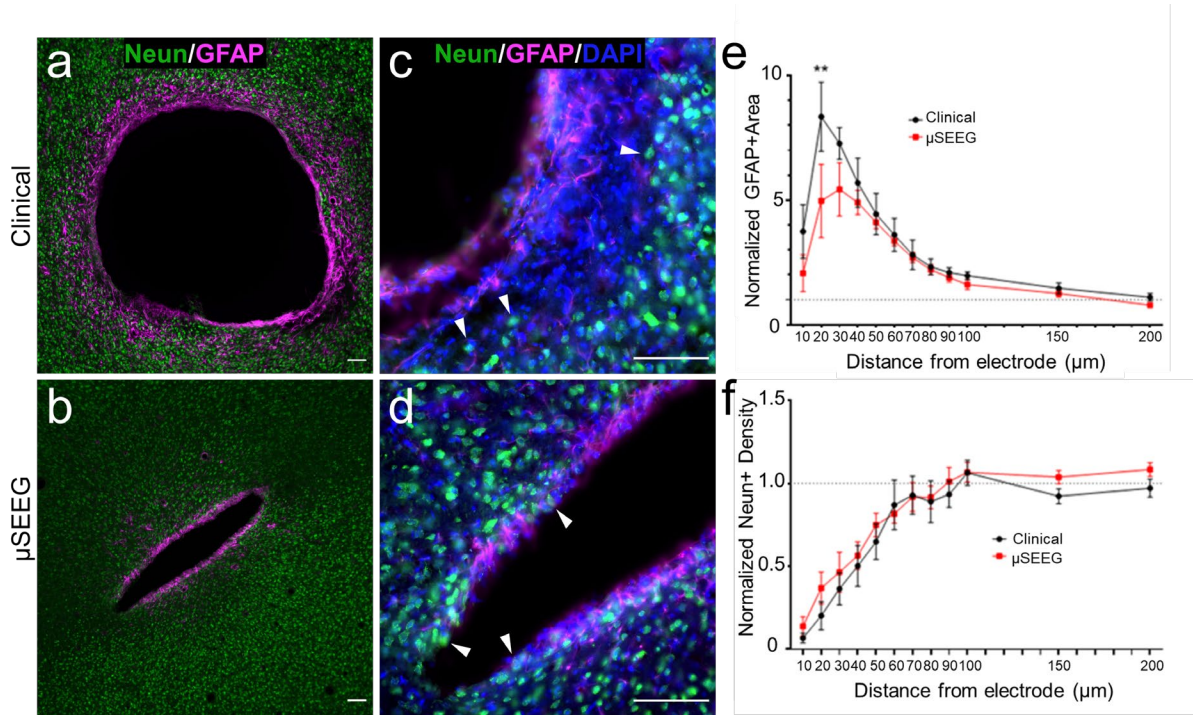
Supplementary Fig. 7. Acute insertion into a phantom brain model. (a) An optical image of the 3D printed holder and the μ SEEG electrode attached to the stereotaxic manipulator. Scale bar is 1 cm. (b) An optical image of the μ SEEG electrode tip inserted in the gelatin model, before and after stylet extraction. Scale bar is 500 μ m.



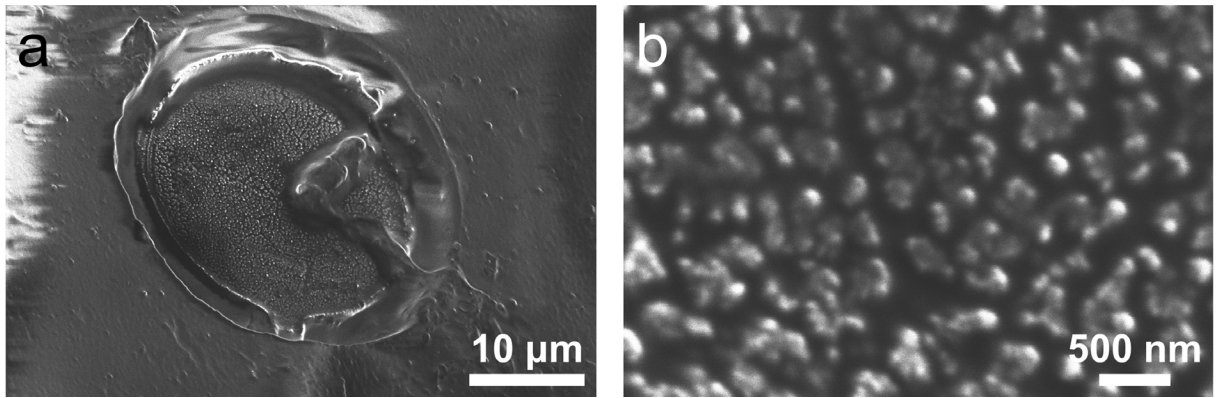
Supplementary Fig. 8. Manufacture and validation of the short PEDOT:PSS μ SEEG in polyimide (PI). (a) A schematic illustration of a prototype 64 channel μ SEEG electrode layout. (b) Exploded view of the layout at the electrode sites region. (c), (d) Optical images of the μ SEEG electrode after stylet insertion. (e), (f) SEM image of μ SEEG electrode showing PEDOT:PSS on Cr/Pt after stylet insertion. (g) Electrochemical impedance spectra of Pt/PEDOT:PSS recording electrodes. (h) Electrochemical impedance magnitude at 1 kHz of each channel before and after stylet insertion and comparison with Pt. (i) averaged impedance magnitude at 1 kHz for 7 PEDOT:PSS μ SEEG electrodes, before and after stylet insertion.



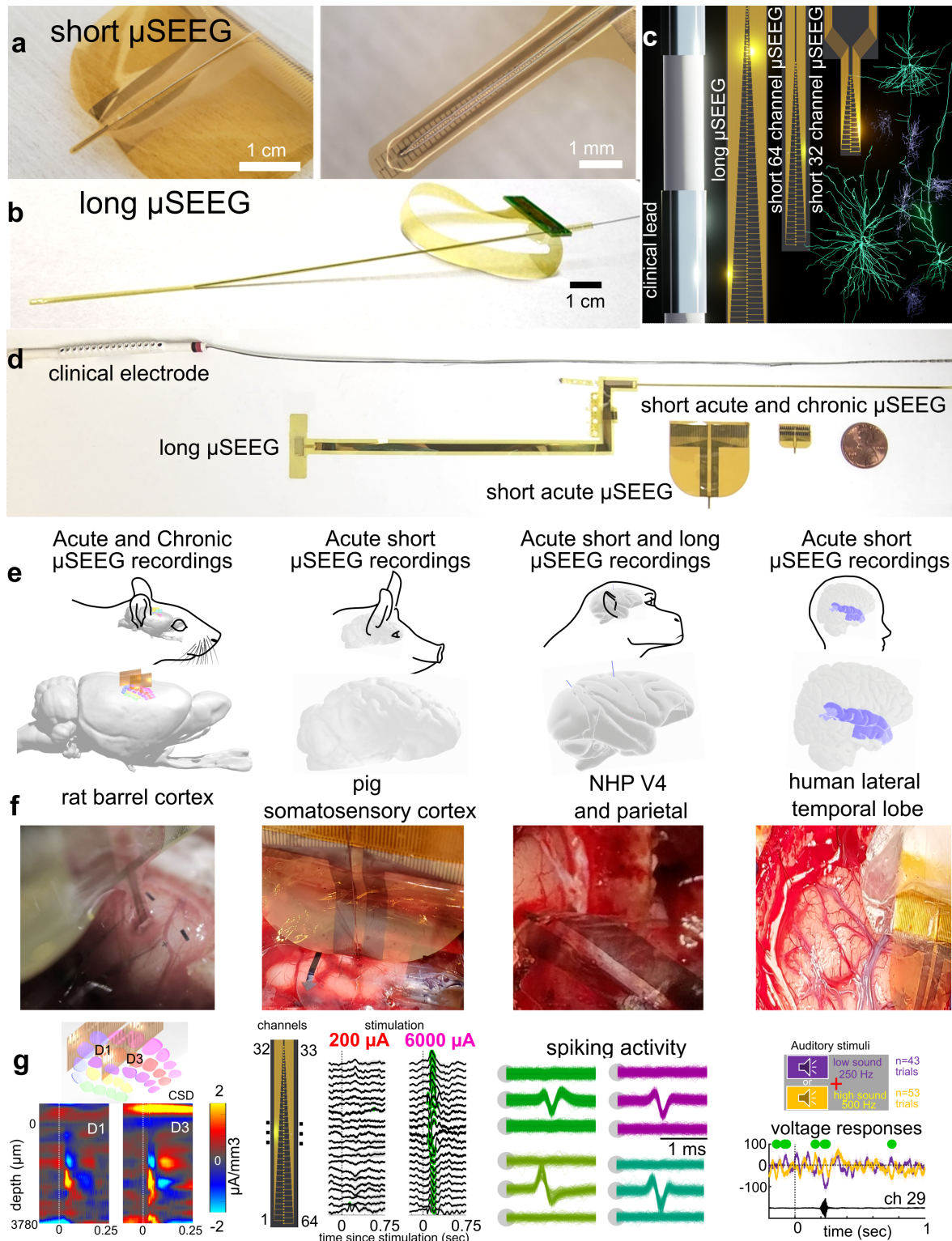
Supplementary Fig. 9. FEM analysis results showing (a) external strength and (b) deformation introduced by stylet insertion. OM images of the PEDOT:PSS/polyimide (c – e) and (f – h) PEDOT:PSS/parylene C electrodes before and after stylet insertion.



Supplementary Fig. 10. GFAP and NeuN reactivity following two weeks of electrode implantation. Wide-field view of horizontal cortical section following (a) clinical or (b) μSEEG electrode implantation. NeuN is pseudocolored green, GFAP magenta, and DAPI blue. (c) and (d) are magnifications of the boxed areas in (a) and (b) to show NeuN reactivity adjacent to electrode placement. Representative NeuN-positive cells are indicated by arrows. (e) Average GFAP area, normalized to each animal (2-way ANOVA, $F(1,72) = 8.749$, $P=0.0042$; $N=4$ /electrode, 1-4 sections per animal). (f) Normalized NeuN+ density (2-way ANOVA, $F(1, 72) = 3.290$, $P=0.0739$, $N=4$ /electrode, 1-4 sections per animal). Data are plotted as mean $\pm$ SEM, **** $P < 0.0001$, ** $P < 0.01$ by 2-way ANOVA followed by Sidak's multiple comparisons test. Scale bar is 100 μm .

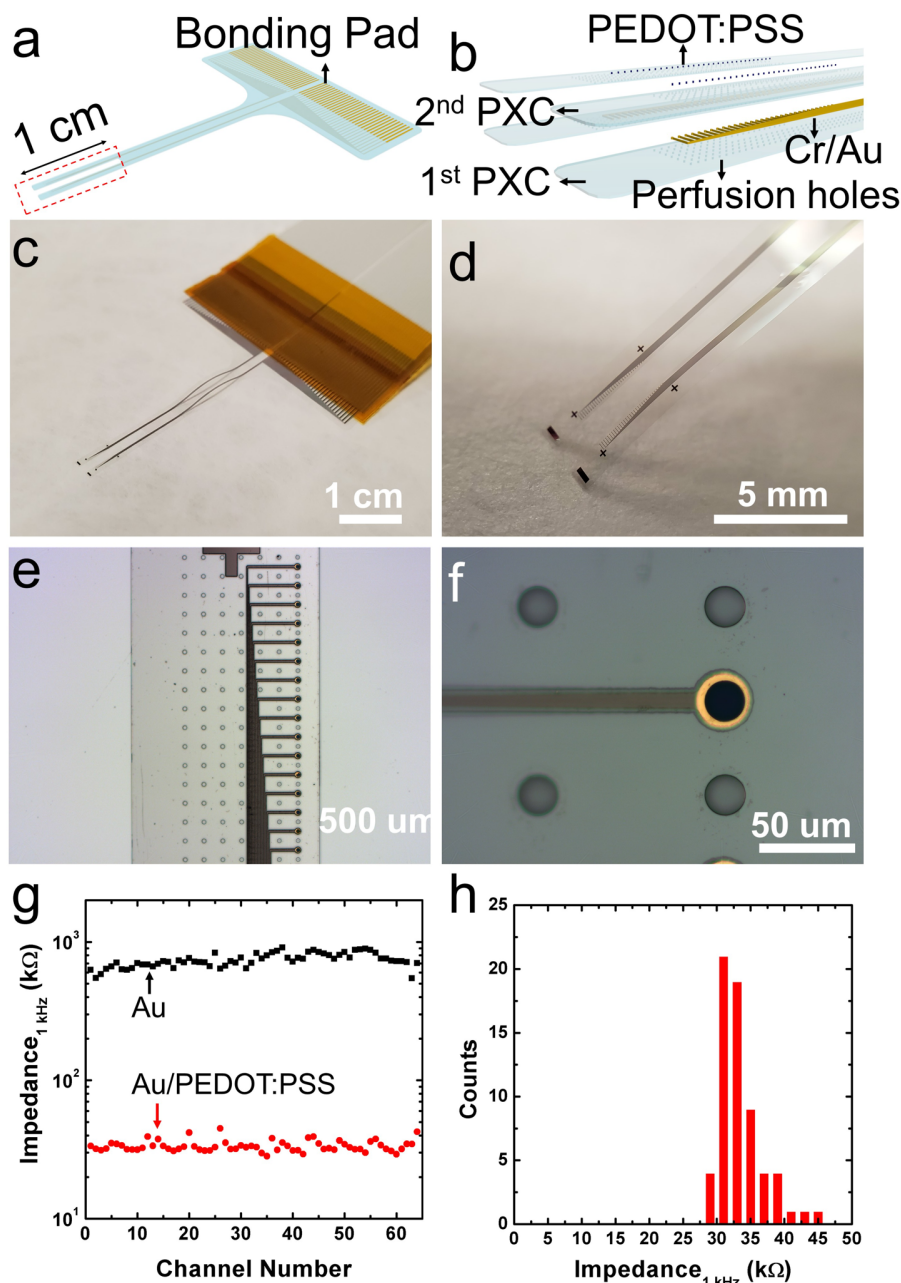


Supplementary Fig. 11. SEM images of μ SEEG electrodes upon extraction. (a) Low magnification image showing whole single PtNRs contact and biological tissue and (b) High magnification image of PtNRs contact.

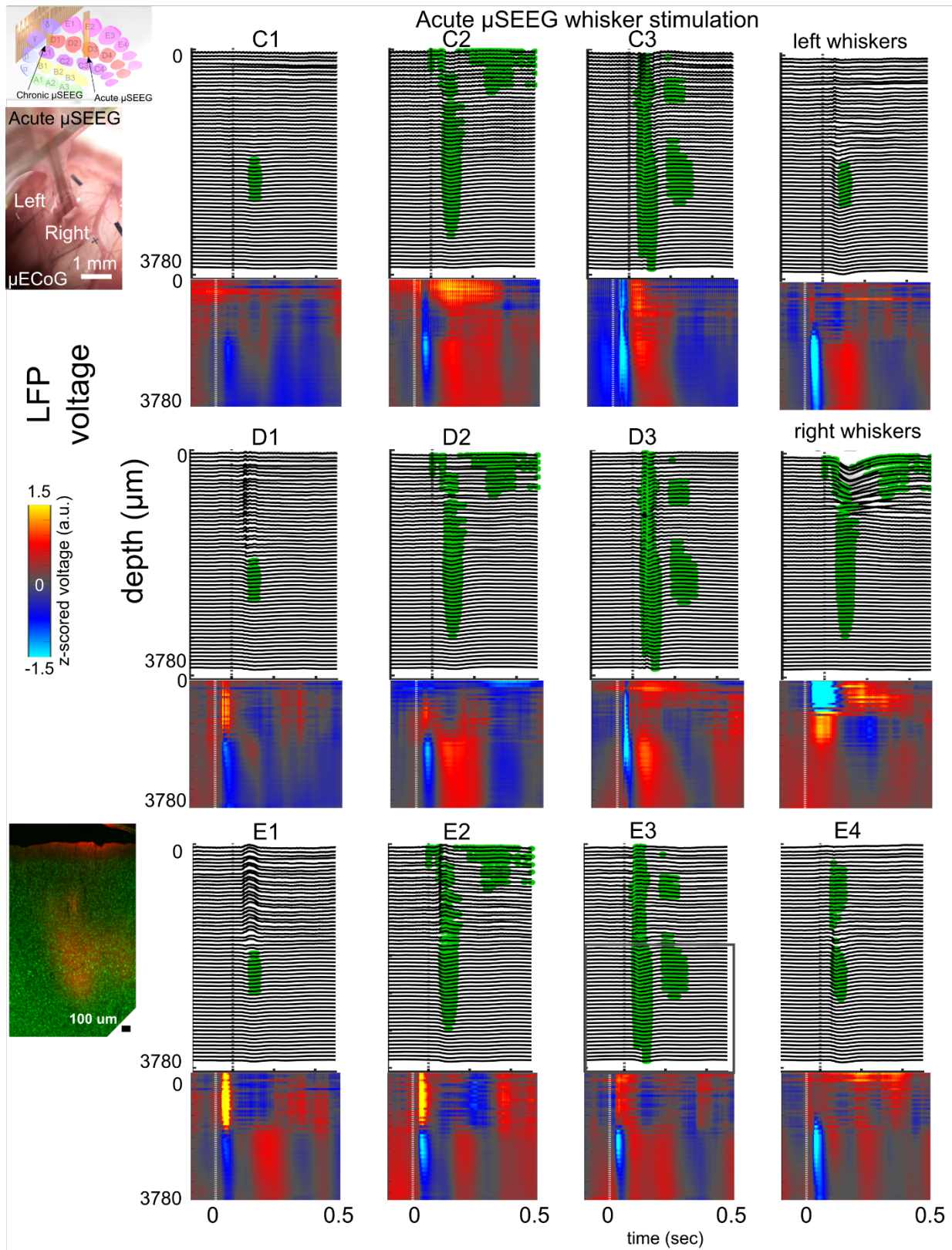


Supplementary Fig. 12. μ SEEG Electrodes for Recording Cortical Neurophysiological Activity in Multiple Species. (a). The short μ SEEG with 64 microelectrode contacts at 60 μ m contact to contact spatial resolution. (b) The long μ SEEG with 128 microelectrode contacts at 60 μ m contact to contact spatial resolution. (c-d) Diagram and photo of the relative scale of human cortical neurons relative to a clinical SEEG lead and a short μ SEEG electrode ²⁵⁻²⁷. (e) Different species and brains

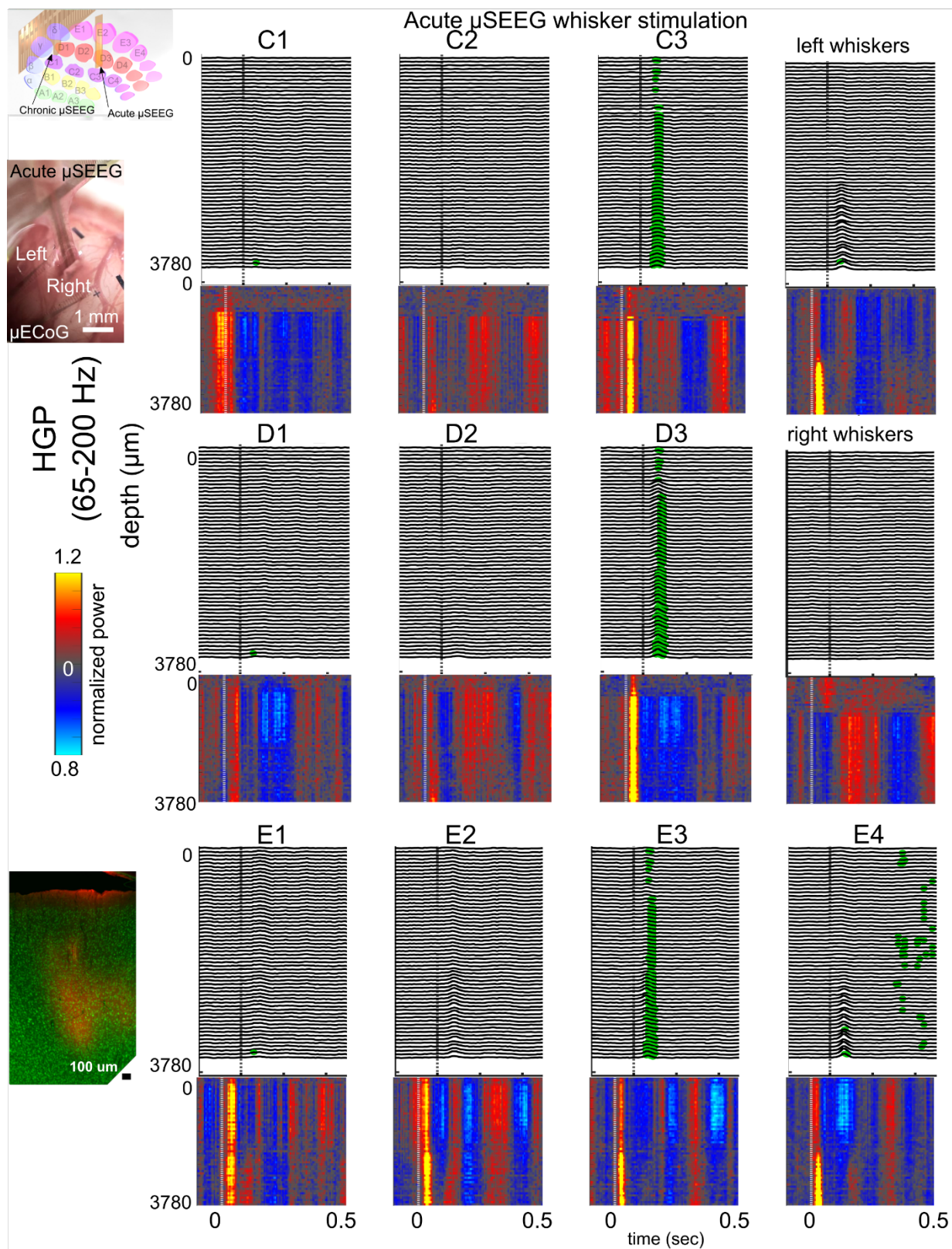
along with the locations of the electrodes for neural recordings in this study. (**f-g**) Images of the different preparations inserted into cortex with example neurophysiological recordings below the images.



Supplementary Fig. 13. Manufacture and characterization of the μ ECoG for comparing surface to depth electrode capabilities. (a) A schematic illustration of μ ECoG electrode layout. Red dotted square indicates electrode sites region. (b) Exploded view of the layout at the electrode sites region. (c), (d) Optical images of the μ ECoG electrode. Scale bar is (c) 1 cm and (d) 5 mm. (e) Top-view optical microscopic image of the electrode sites region. (f) Magnified top-view optical microscopic image showing PEDOT:PSS on Cr/Au electrode sites and perforation holes. (g) Electrochemical impedance magnitude at 1 kHz of each channel of μ ECoG electrode before and after PEDOT:PSS electrodeposition. (h) Electrochemical impedance magnitude at 1 kHz histogram of Au/PEDOT:PSS.



Supplementary Fig. 14. Acute laminar voltage recordings in the rat barrel cortex with the μ SEEG with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of the implanted μ ECoG electrode (left) and the μ SEEG electrode (right). First, the μ ECoG electrode was placed on the cortical surface. At the gap between two columns of the μ ECoG electrode, the μ SEEG electrode was implanted, and the stylet was removed using the previously described technique. Note some contacts are outside brain tissue on the μ SEEG electrode. Voltage responses across the μ SEEG electrode to whisker stimulation at different whisker locations for the voltages (local field potential or LFP). Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.

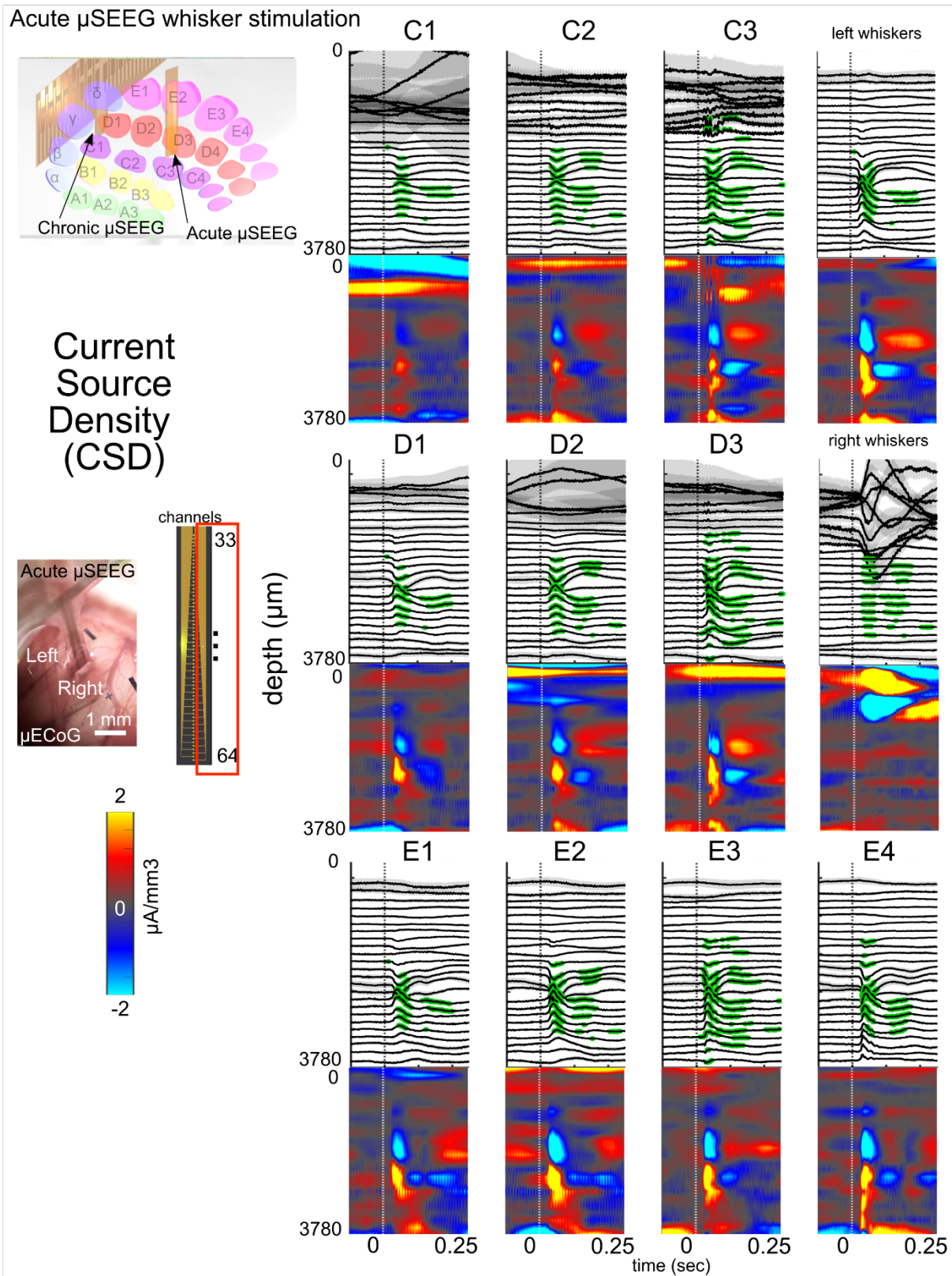


Supplementary Fig. 15. Acute laminar high gamma power recording in the rat barrel cortex with the μ SEEG with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of the implanted μ ECoG

electrode (left) and the μ SEEG electrode (right). First, the μ ECoG electrode was placed on the cortical surface. At the gap between two columns of the μ ECoG electrode, the μ SEEG electrode was implanted, and the stylet was removed using the previously described technique. Note some contacts are outside brain tissue on the μ SEEG electrode. Responses across the μ SEEG electrode to whisker stimulation at different whisker locations for the high gamma power (HGP). Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.

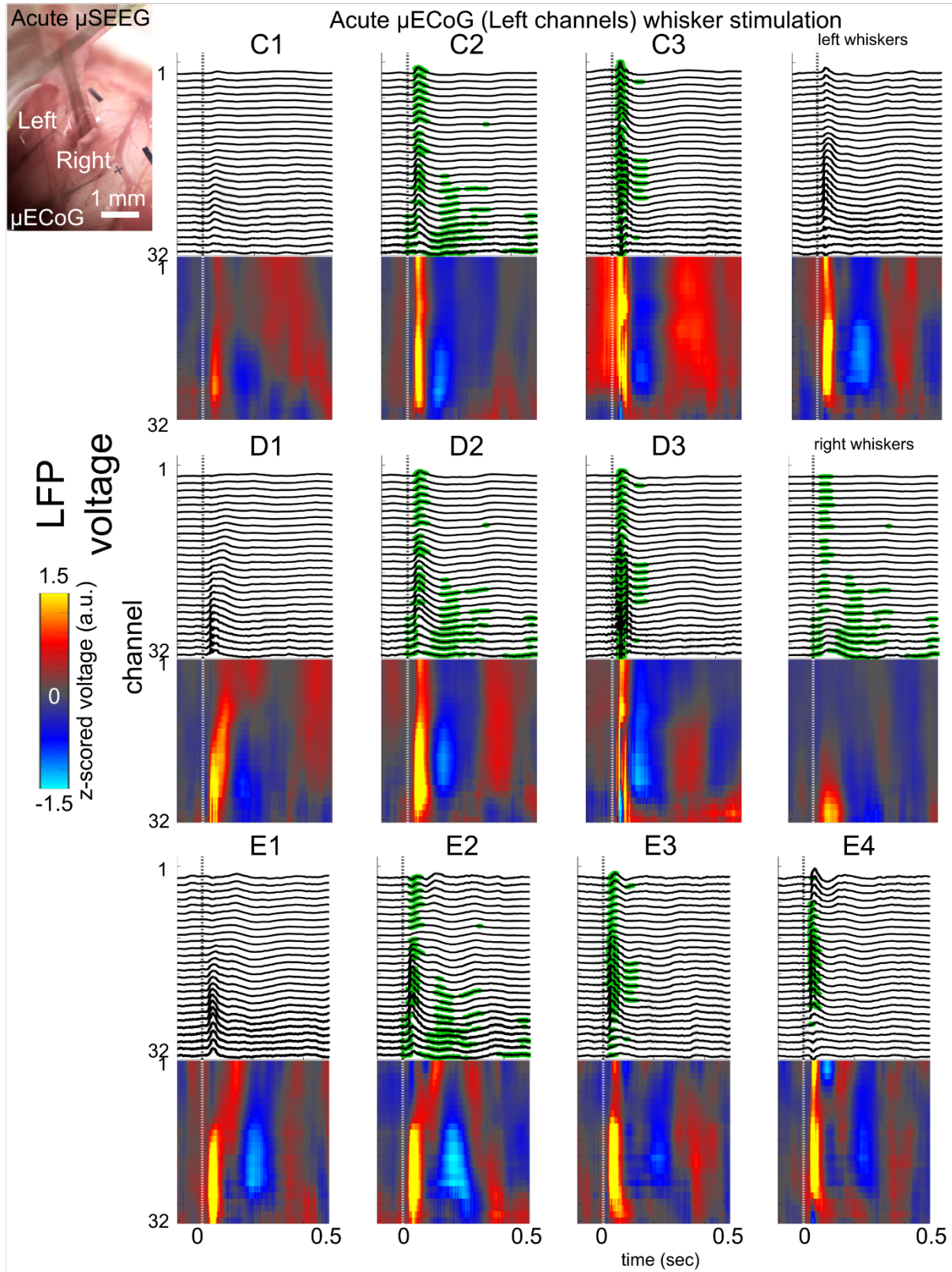


and chronic implantation of μ SEEG electrodes d for recording from the rat barrel cortex and images of the implanted surface μ ECoG electrode and the μ SEEG electrode. Note some contacts are outside brain tissue on the μ SEEG electrode. Current source density (CSD) responses across half the μ SEEG electrode (left side) to whisker stimulation at different whisker locations. Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.



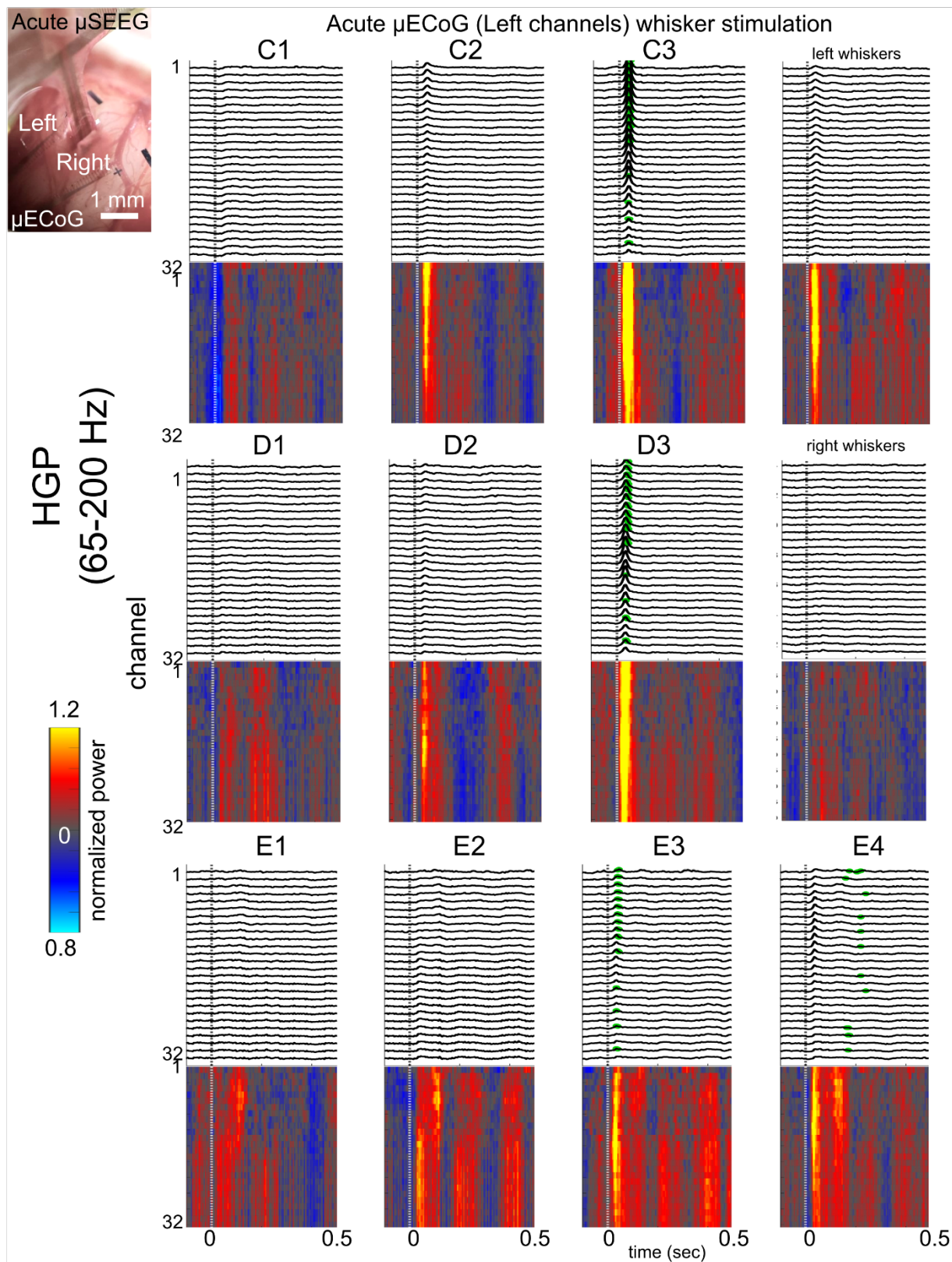
Supplementary Fig. 17. Acute laminar recording of current source density (CSD) for the rightmost channels in the rat barrel cortex with the μ SEEG with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes d for recording from the rat barrel cortex

and images of the implanted surface μ ECOG electrode and the μ SEEG electrode. Note some contacts are outside brain tissue on the μ SEEG electrode. Current source density (CSD) responses across half the μ SEEG electrode (right side) to whisker stimulation at different whisker locations. Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.



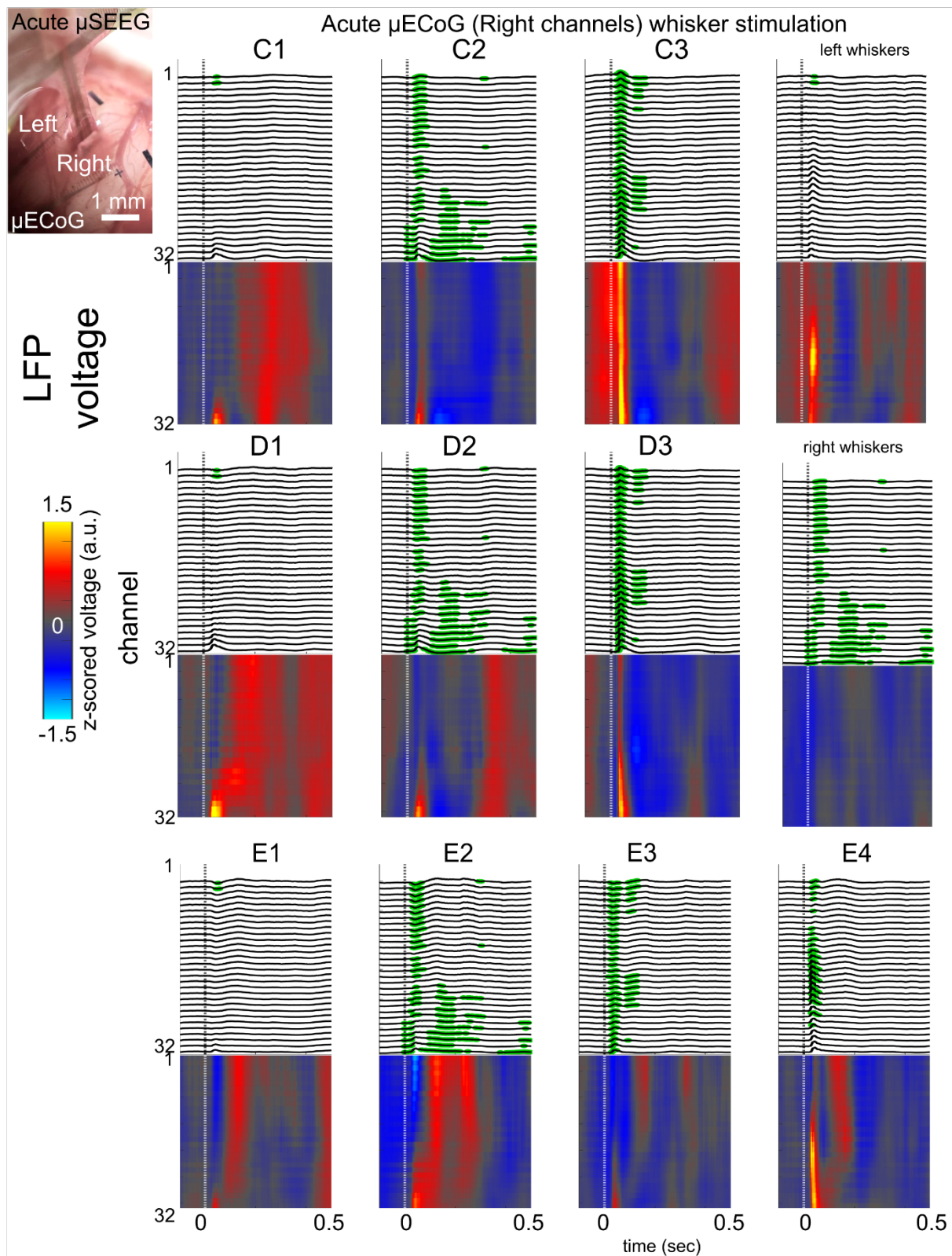
Supplementary Fig. 18. Acute surface local field potential (LFP) voltage recording with a strip of the μ ECoG over the rat barrel cortex with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of

the implanted μ ECoG electrode (left) and the μ SEEG electrode (right). Note some contacts are outside brain tissue on the μ SEEG electrode. Voltage responses across the left ribbon of the μ ECoG electrode to whisker stimulation at different whisker locations for the voltages and multi-unit activity (MUA). Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.



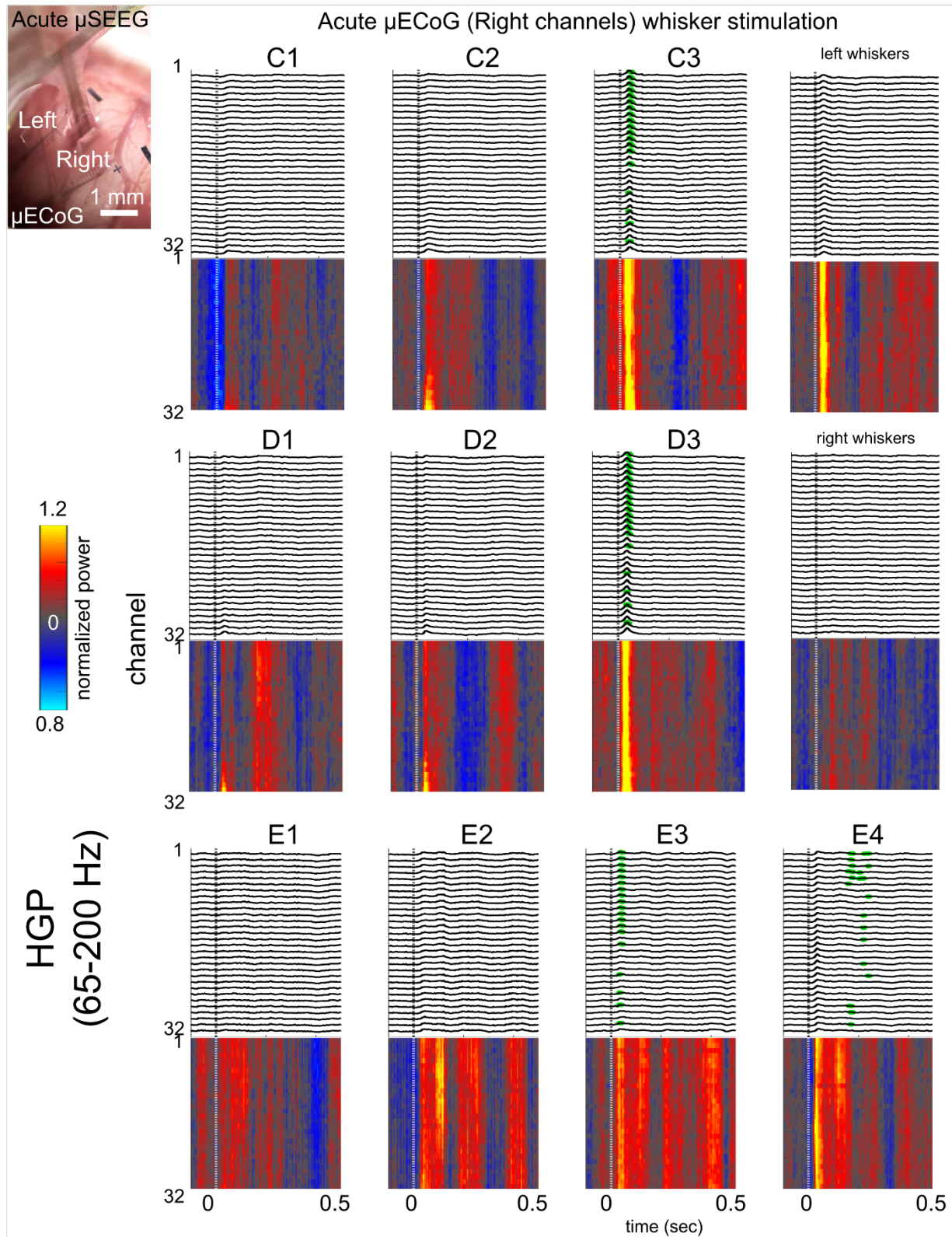
Supplementary Fig. 19. Acute surface high gamma power (HGP) recording with a strip of the μ ECoG over the rat barrel cortex with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of the implanted

5 μ ECoG electrode (left) and the μ SEEG electrode (right). Note some contacts are outside brain tissue on the μ SEEG electrode. Responses across the left ribbon of the μ ECoG electrode to whisker stimulation at different whisker locations for the high gamma power (HGP). Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.



Supplementary Fig. 20. Acute surface local field potential (LFP) voltage recording with a strip of the μ ECoG over the rat barrel cortex with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of

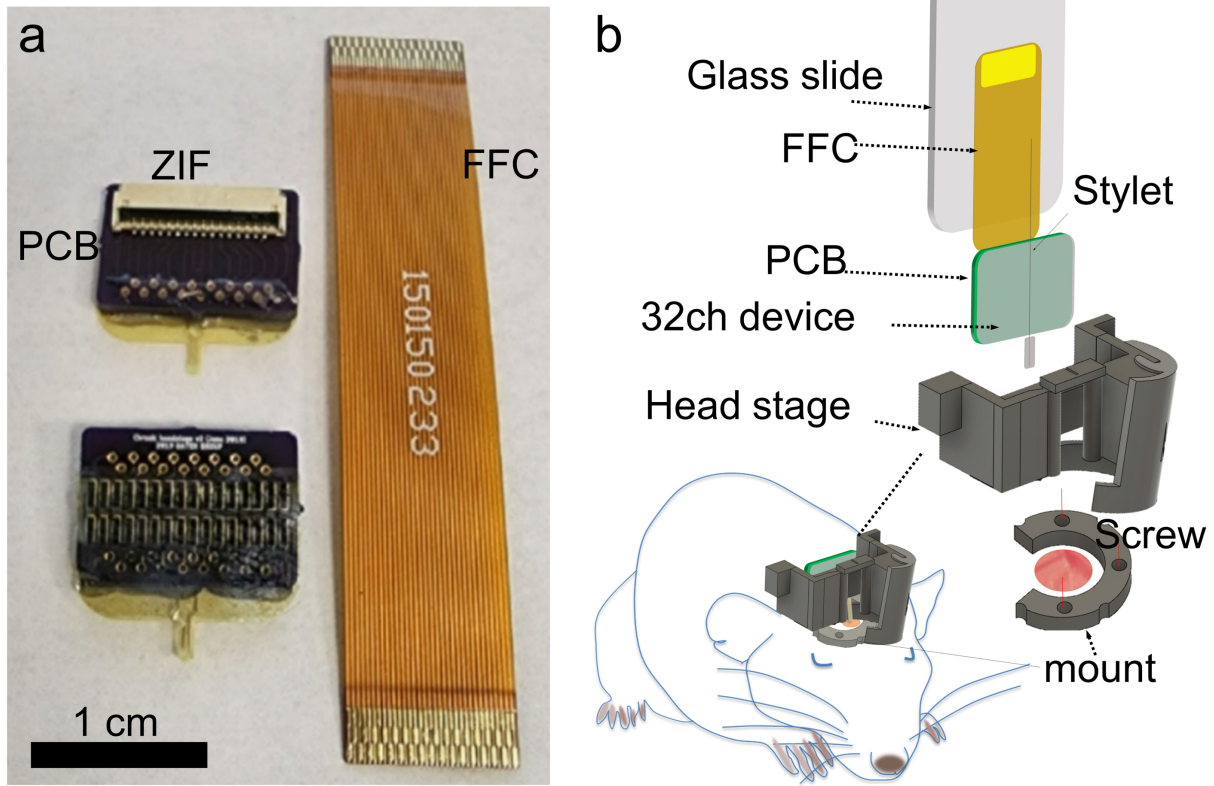
the implanted μ ECoG electrode (left) and the μ SEEG electrode (right). Note some contacts are outside brain tissue on the μ SEEG electrode. Voltage responses across the right ribbon of the μ ECoG electrode to whisker stimulation at different whisker locations for the voltages. Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.



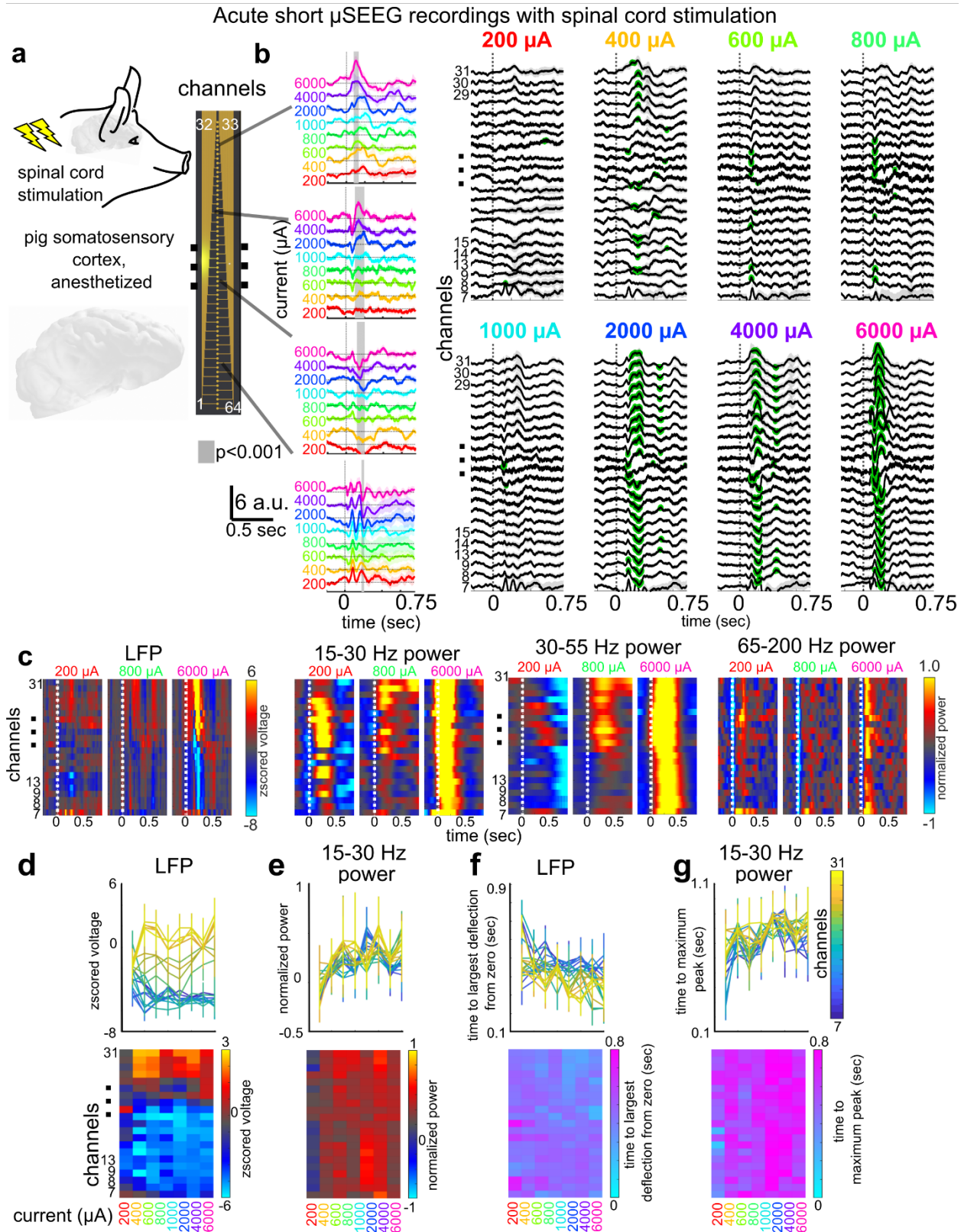
Supplementary Fig. 21. Acute surface high gamma power (HGP) recording with a strip of the μ ECoG over the rat barrel cortex with sensory stimulation. Upper left: Location and 3D reconstruction of possible locations of the acute and chronic implantation of μ SEEG electrodes for recording from the rat barrel cortex and images of the implanted

5 μ ECoG electrode (left) and the μ SEEG electrode (right). Note some contacts are outside brain tissue on the μ SEEG electrode. Responses across the right ribbon of the μ ECoG electrode to whisker stimulation at different whisker locations for the voltages and multi-unit activity (MUA). Green dots indicate significantly different from 0.5 sec before (which is baseline) air puff stimulation to the whisker (Wilcoxon rank-sum test) per channel and across trials. Number of trials >10.

10

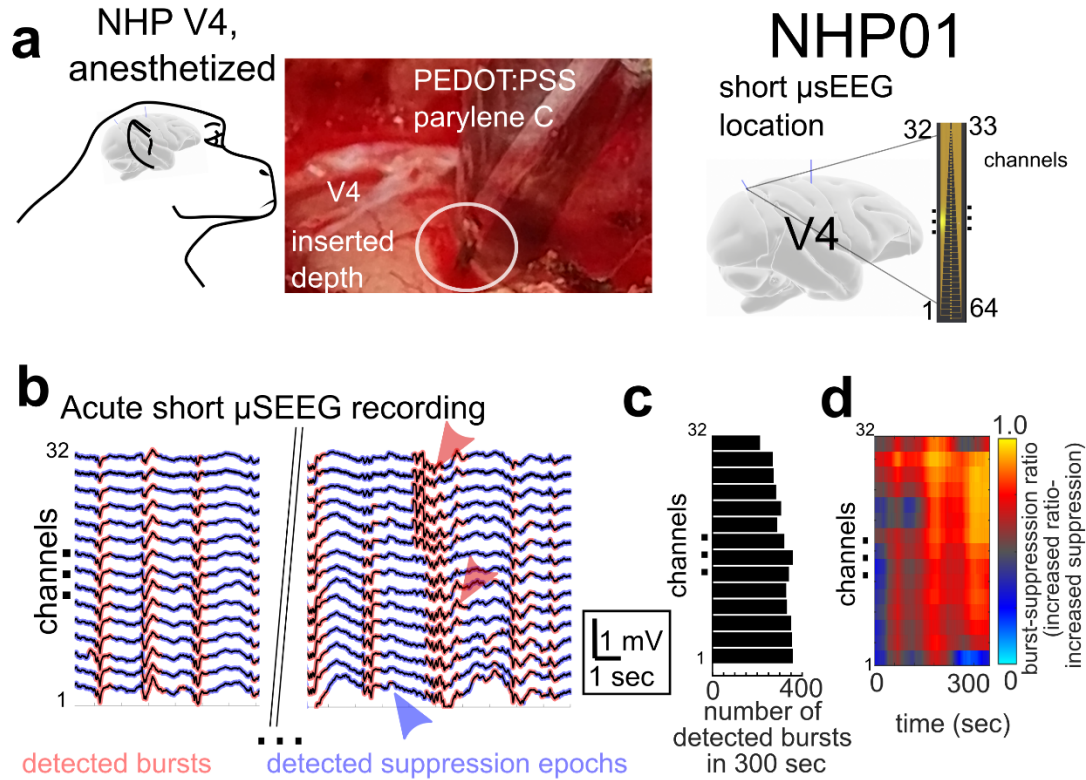


Supplementary Fig. 22. Chronic implantation of the μ SEEG electrode and recordings. (a) Hardware components and (b) Illustrations of the chronic μ SEEG electrode implantation procedures.

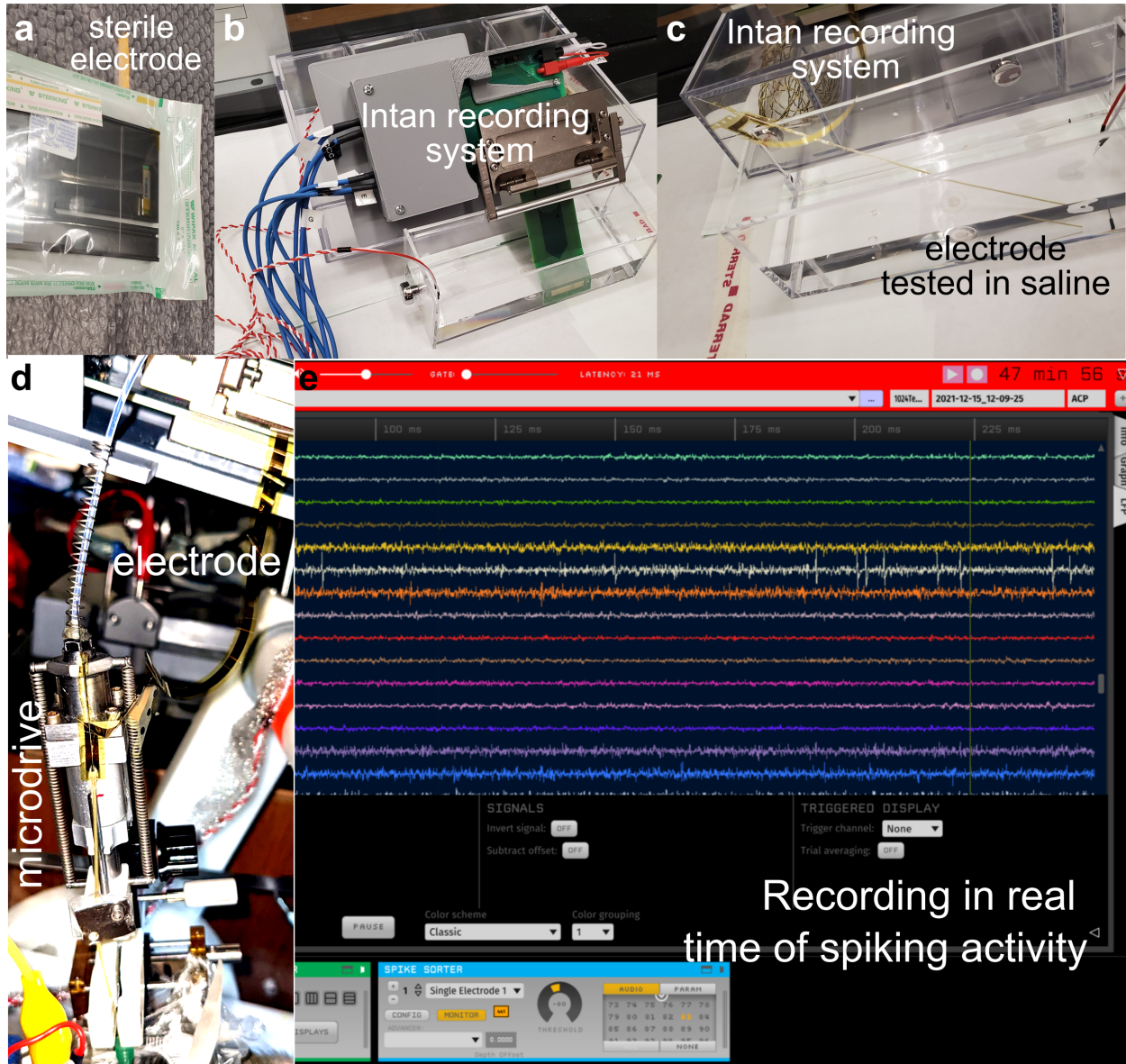


Supplementary Fig. 23. Acute short 64 channel μ SEEG recordings in an anesthetized pig. (a) Direct electrical stimulation of the spinal cord while doing an acute short 64 channel μ SEEG electrode recording from the pig cortex, with responses increasing with increasing injected current. Grey bar indicates significantly different

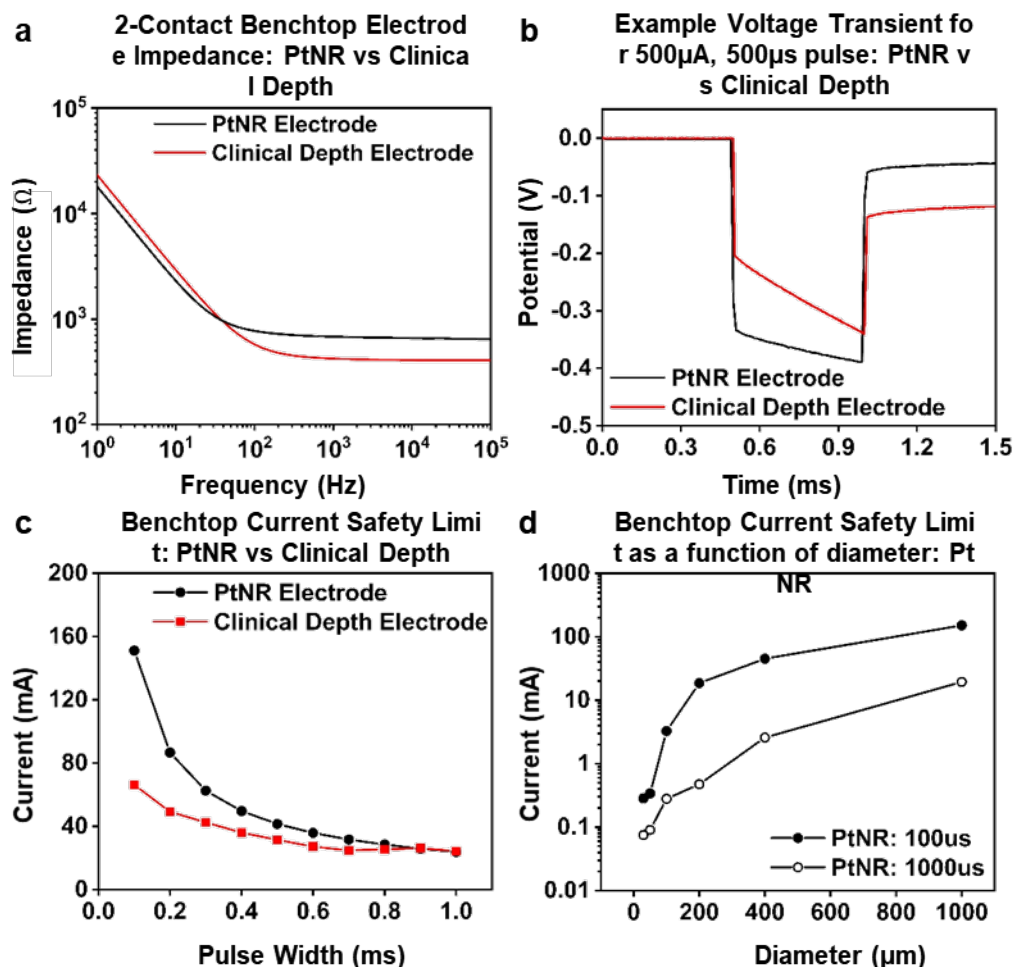
between current steps, Wilcoxon rank sum test. **(b)** Voltage responses along the electrode depth with more responses significantly different to baseline (0.5 sec before stimulation) occurring more with higher current levels (green dots, Wilcoxon rank sum test). **(c)** Average responses to stimulation in different frequency bands to different levels of injected current across channels. **(d)** Top: zscored voltage and Bottom: Two dimensional map of the time to zscored voltage per channel and per current step. **(e)** Top: 15-30 Hz power and Bottom: Two dimensional map of the time to 15-30 Hz power per channel and per current step. **(f)** Top: time to peak voltage and Bottom: Two dimensional map of the time to peak deflection from baseline per channel and per current step **(g)** Top: time to maximum deflection in 15-30 Hz power and Bottom: Two dimensional map of the time to maximum deflection in 15-30 Hz power per channel and per current step.



Supplementary Fig. 24. Acute short 64 channel μ SEEG recordings in an anesthetized non-human primate (NHP). (a) Acute implant of the short μ SEEG electrode into V4 in an anesthetized NHP and ongoing evidence of burst suppression detected using automated approaches per channel. Arrowheads indicate detected bursts. (b) Number of detected bursts in 300 seconds per channel, with a correlation between channel depth and the number of bursts $r=0.72$; $p=0.0021$ (Pearson's linear correlation). (c) burst-suppression ratio changes over time during the recording across channels.



Supplementary Fig. 25. Set up and Microdrive recording using the long μ SEEG electrode to record ongoing activity. (a) Sterile electrode used in recording, sterilized using Ethylene Oxide gas. (b) 1024 channel Intan system used to record activity, testing in saline. (c) Testing of the long μ SEEG electrode in saline. (d) Microdrive used to deliver the microelectrode. The same microdrive was used to drive tungsten electrodes to record activity. (e) Recording of real-time spiking activity using the long μ SEEG electrode.



Supplementary Fig. 26. Electrochemical characterization of an AdTech Medical Spencer depth that is 1.12 mm diameter with contacts that are 2.41 mm long and 5 mm spaced and a PtNR electrode with 1 mm diameter during stimulation. (a) Two-contact electrochemical impedance spectra (where another contact is used as both ground and reference to simulate impedances that appear in bipolar stimulation). The PtNR contact with 28.8 times smaller area than the PMT electrode exhibited higher capacitance and therefore lower reactance than the PMT electrode. The 1 kHz impedance that is typically overwhelmed with series resistances of metallization traces for macro-contacts is larger of the PtNR contact than the PMT electrode. (b) Example voltage transient across the two types of electrodes, exhibiting a lower potential drop across the electrode-tissue interface (non-instantaneous potential drop) for the PtNR contact compared to the PMT depth contact. (c) Benchtop electrochemical safety limits for the two types of electrodes when tested in the bipolar configuration (see ref. ²⁸ for methods), showing superior performance for the PtNR contact, particularly at small pulse widths. (d) The benchtop electrochemical safety limits for two pulse widths for the PtNR contacts as a function of contact diameter.

Supplementary Video 1. Movement of the implanted short thin film μ SEEG relative to the brain. The electrode is shown before and after insertion, where the short depth was inserted into brain tissue and activity was recorded for a short period of time. The gauze and other stabilizing features are largely for the ribbon cables which form the connections, but the thin film components (which are largely transparent and flexible) move with the brain tissue movements in the craniotomy.

Supplementary Table 1. Comparison of the electrode form factor with previously reported studies using rolled solutions. (D: diameter, Parylene: Parylene C, EIROF: electrodeposited iridium oxide film, PEDOT: PEDOT:PSS, PtNR: Platinum nanorods.)

	Total channel count	Metal lead spacing	Electrode site size & material	Impedance (k Ω) @ 1 kHz	Probe material	Probe shape	Subject, implant site
van der Puije et al. (1989)	9	N/A	890 μm x (1370 ~ 2180 μm), Pt	~ 1	Polyimide/silicone rubber	Rolled (cylindrical)	N/A (benchtop)
Kang et al. (2015)	20	N/A	D: 100 μm , EIROF on Pt	1.941 - 2.026	Parylene	Rolled (self-closed)	Rat, sciatic nerve
Pothof et al. (2016)	32, 64 (prototype: 128)	N/A	D: 35 μm , Pt	354 $\pm$ 31	Polyimide	Rolled (cylindrical)	NHP, cortex
This work (2023), PEDOT	32, 64	3 μm	D: 20 μm , PEDOT on Pt	35.0 $\pm$ 3.7	Parylene, polyimide	Inflatable sheath	Rat/NHP/pig/human, cortex
This work (2023), PtNR	32, 128	3 μm	D: 30 μm , PtNR	94.0 $\pm$ 2.6	Polyimide	Inflatable sheath	Rat/NHP, cortex

Supplementary Table 2. Electrode usage and species tested. Sp. – species, NHP- Non-Human primate, PEDOT-PEDOT:PSS, PI-Polyimide, PC-Parylene C.

label	Sp.	Brain region	Electrode	PEDOT or PtNR	PI or PC	Acute or chronic
HS1	Human	Left anterior temporal lobe	Short 64 channel μ SEEG	PEDOT	PC	Acute
HS2	Human	Left anterior temporal lobe	Short 64 channel μ SEEG	PEDOT	PI	Acute
MM1	NHP	Visual cortex	Short 64 channel μ SEEG	PEDOT	PC	Acute
MM1	NHP	Visual cortex	Surface μ ECoG	PtNR	PC	Acute
MM1	NHP	Visual cortex	Surface μ ECoG	PEDOT	PC	Acute
MM2	NHP	Parietal lobe/dlPFC and auditory cortex	Long μ SEEG	PtNR	PI	Acute
MM2	NHP	Parietal lobe/dlPFC and auditory cortex	Tungsten electrode	-	-	Acute
RN1	Rat	Near barrel cortex	Short 32 channel μ SEEG	PEDOT	PI	Chronic
RN2	Rat	Near barrel cortex	Short 32 channel μ SEEG	PEDOT	PI	Chronic
RN3	Rat	Near barrel cortex	Short 32 channel μ SEEG	PEDOT	PI	Chronic
RN4	Rat	Near barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic
RN5	Rat	Near barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic
RN6	Rat	Near barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic
RN7	Rat	Near barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic

RN8	Rat	Barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic
RN9	Rat	Barrel cortex	Short 32 channel μ SEEG	PtNR	PI	Chronic
RN10	Rat	Near barrel cortex	Short 64 channel μ SEEG	PEDOT	PI	Acute
RN10	Rat	Near barrel cortex	Surface μ ECoG	PEDOT	PC	Acute
SS1	Pig	Brain	Short 64 channel μ SEEG	PEDOT	PC	Acute
SS2	Pig	Brain	Short 64 channel μ SEEG	PEDOT	PI	Acute
SS2	Pig	Brain	Surface μ ECoG	PEDOT	PC	Acute
SS3	Pig	Brain	Short 64 channel μ SEEG	PEDOT	PI	Acute
SS3	Pig	Brain	sEEG electrode	-	-	Acute
SS3	Pig	Brain	Surface μ ECoG	PEDOT	PC	Acute

Supplementary Table 3. Electrode details.

Electrode	Number of channels	Diameter (μ m)	Center to center spacing (μ m)	Recording length (mm)	PEDOT:PSS or PtNR	Polyimide (PI) or parylene C (PC)
Short 32 channel μ SEEG	32	30	60	1.89	PEDOT:PSS, PtNR	PI
Short 64 channel μ SEEG	64	20	60	3.80	PEDOT:PSS	PI, PC
Long μ SEEG	128	30	60	7.65	PtNR	PI
Surface μ ECoG	32 x 2	20	100	3.12	PEDOT:PSS	PC

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