nature protocols

https://doi.org/10.1038/s41596-023-00871-2

Protocol

Check for updates

Modified Neuropixels probes for recording human neurophysiology in the operating room

In the format provided by the authors and unedited

Supplementary Information for

Title: Use of modified Neuropixels probes for recording human neurophysiology in the operating room

Author List

Brian Coughlin[1]*, William Muñoz[2]*, Yoav Kfir[2]*, Michael J. Young[1], Domokos Meszéna[1], Mohsen Jamali[2], Irene Caprara[2], Richard Hardstone[1], Arjun Khanna[2], Martina L. Mustroph[3], Eric M. Trautmann[4], Charlie Windolf[6], Erdem Varol[7], Dan J. Soper[1], Sergey D. Stavisky[5], Marleen Welkenhuysen[8], Barundeb Dutta[8], Krishna V. Shenoy[9], Leigh R. Hochberg[1,10], R. Mark Richardson[2], Ziv M. Williams[2]‡, Sydney S. Cash[1]‡, and Angelique C. Paulk[1]‡

Supplementary Materials

Supplementary Figure 1. Recording system and set up.

Supplementary Figure 2. Custom cable schematics and examples.

Supplementary Figure 3. Pre-procedure day noise testing in OR and effect of different overhead light sources on Neuropixels recordings.

Supplementary Figure 4. Open Ephys Software and set up.

Supplementary Figure 5. Messages and information using SpikeGLX.

Supplementary Table 1. Data processing parameters per processing step

Supplementary Video 1. Ongoing human brain neural activity recorded via Neuropixels using SpikeGLX and OpenEphys recording software, as recorded in the operating room.

Supplementary Video 2. Demonstration of the additional electrical noise from the wall-powered anaesthesia pump through the a saline tube while recording in saline and gelatine.

Supplementary Video 3. Setting up the Neuropixels probe in the sterile field to be connected to the recording system.

Recording system and set up

Recording system and set up (**Supplementary Figure 1**) shows all the components of the recording system and the connections used in the recording system including task-related hardware.

Custom Cabling: Making Parallel to BNC (Octopus) Cables

These cables are created to enable trigger signals to allow for synchronization across systems (**Supplementary Fig. 2**).

1. Cut four ~60cm BNC cables in half - longer cables are fine, but ~30cm or slightly shorter is ideal to keep cables from being too unwieldy.
2. Use a razor to strip the ends of each cable approx. 2.5cm to reach the inside wires (keep both the signal [center] wire and shielding intact).
3. Cinch the shielding wire (mesh) down to expose the insulation covering the signal wire.
4. Strip the insulation for the signal wire, but keep enough so that the shielding and signal wires will not touch easily.
5. Solder the signal (center) wire of each BNC onto the top pins 2-9. When looking at the back of the connector, with the longer row on top, pin 1 is on the top left. Double check if needed with numbers that are usually printed on the front plastic face of the connector.

Denoising Neural Recordings

An ongoing challenge with any recordings in an operating room is that it is a relatively uncontrolled environment regarding electrical noise. Ambient electrical noise coming from the numerous devices used in the course of the surgery range from anesthesia machines (**Supplementary Video 2**), powered surgical tools, devices for brain visualizations and neuronavigation (BrainLab), ultrasound devices, robotic surgical tools (ROSA ONE® Brain -

Zimmer Biomet- robot), light sources, powered surgical beds, and powered thermal body and leg warmers. All of this can vary considerably with different operating rooms, some of which are equipped with moveable MRI or CT machines. Not all of these operating room devices are grounded to a common ground and the Neuropixels probe is sensitive to both electrical and visual (flickering) light (**Supplementary Fig. 3**). Therefore, noise represents a major obstacle to detecting single neuron activity using Neuropixels. To best accommodate for these noise sources, we tested several grounding and referencing schemes[1]. In our case, a critical step in reducing overall noise levels was to separate the ground and reference (in contrast to the generally proposed solution in rodent studies which involved tying them together [2]). We used the external reference tied to a sterile needle electrode inserted into the nearby skin or muscle with the ground tied to a separate sterile needle electrodes inserted into a second location (**Fig. 1**). Using the internal reference option resulted in considerably increased noise levels[1]. As to external noise sources, we found the major source in our case was the wall-powered anesthesia intravenous pump (as is frequently used during patient transport), which, when unplugged and operating on battery, would decrease the physiological as well as the common 60 Hz noise. Otherwise, we have not experienced any noticeable effect on noise when turning off other medical devices (BOVIE cautery machine, ROSA robot, AlphaOmega recording system, etc.) but these findings may be site- and case-specific. The Neuropixels probe contains a number of transistors sensitive to light flicker [2,3] which is present in certain OR lights (to variable degrees), but not in others. This issue was resolved when we asked the overhead OR lights be turned off during the recordings and have the field lit by a battery-powered headlamp. In conclusion, each external noise source should be investigated individually by the experimenters per site to find an optimal implantation and recording strategy. If possible, testing these devices in the OR without a patient in the room with the Neuropixels probe in saline can provide an excellent testing set up for identifying the best noise levels as needed (**Supplementary Figure 3; Supplementary Video 1**).

Interfacing with the recording software: SpikeGLX and OpenEphys

We recommend the use of both OpenEphys[4] (**Supplementary Figure 4**) and SpikeGLX (**Supplementary Figure 5; Supplementary Video 1**). SpikeGLX enables easy testing of the probe (**Supplementary Figure 5**) while OpenEphys currently allows better data visualization and considerable flexibility including online spike sorting. The instructions below assume some familiarity with the documentation associated with the software. It is recommended that users review the documentation for both Open Ephys and SpikeGLX as the documentation is quite informative in terms of Neuropixels use. With both OpenEphys and SpikeGLX, channel maps are needed to select the 384 contacts used to record out of all contacts on the Neuropixels probe. Channel maps are included with the software downloads. Also in both systems, the signal is acquired as local field potential (LFP, <500 Hz filtered data, sampled at 2500 Hz) and action potential (AP, >500 Hz filtered data, sampled at 30000 Hz) bands. This cannot be altered in the initial acquisition though can be filtered or downsampled further in OpenEphys if needed (please see OpenEphys documentation). We preferentially directly recorded these signals to be saved to file along with the TTL pulses from the separate NIDAQ base station (see below and the Protocol).

Open Ephys (ver 0.6.0): https://open-ephys.org/gui

SpikeGLX (Release_v20221012-phase30): https://billkarsh.github.io/SpikeGLX/

1. Ensure Neuropixels calibration files (2 files per probe) are copied to C:\Program Data\Open Ephys\CalibrationInfo\<probe_serial_number>
   One file should be “<probe serial number>_ADCCalibration.csv”
   The other file should be “<probe serial number>_gainCalValues.csv”
   Open Ephys will be able to locate calibration files in this directory automatically. A copy

of each should also be placed in <SpikeGLX installation directory>\SpikeGLX_Calibration\<probe serial number> for SpikeGLX use.

2. Ensure IMRO map files are in a known location and load properly when a Neuropixels probe is attached. IMRO maps are loaded through the Neuropixels-PXI plugin. Have at least one IMRO file use a tip reference and one use an external reference to best prepare for denoising processes in OR. A “long” map which uses channels along the entire length of the probe can be useful for estimating probe depth in the cortex.
3. Set up a signal chain in Open Ephys to be used for the OR recording (**Supplementary Figure 4**). A should be the Neuropixels-PXI plugin. Source B should be the NI-DAQmx plugin. Place visualization plugins (probe viewer; LFP viewer; spike detector + spike viewer; audio monitor) downstream from the record node.

   CAUTION: There is one mode in OpenEphys where you can use a Merger plugin to merge the IMEC and NIDAQ streams of data. We attempted to use this mode but found that, if there were brief pauses in acquisition, the data was not acquired correctly or synchronized. Therefore, we recommend keeping the streams of data separate (IMEC and NIDAQ) and synchronizing through post-processing using a TTL pulse being sent to both systems simultaneously.
4. Test synchronization of data streams by sending triggers from MATLAB PC split to both the I/O module (BNC 2110) and IMEC PXIe boards via parallel port (through parallel to BNC connector). Ensure record node plugin shows green boxes on the sync monitor below the continuous data buffer monitor bars. (**Supplementary Figure 4**)

   ?TROUBLESHOOTING
5. Select write path on record node and make sure there is adequate disk space for the recording
6. Select “Binary” as the recording engine from the dropdown menu on the record node

7. Ensure “RECORD EVENTS” box is selected (red with black dot)

Electrode localization

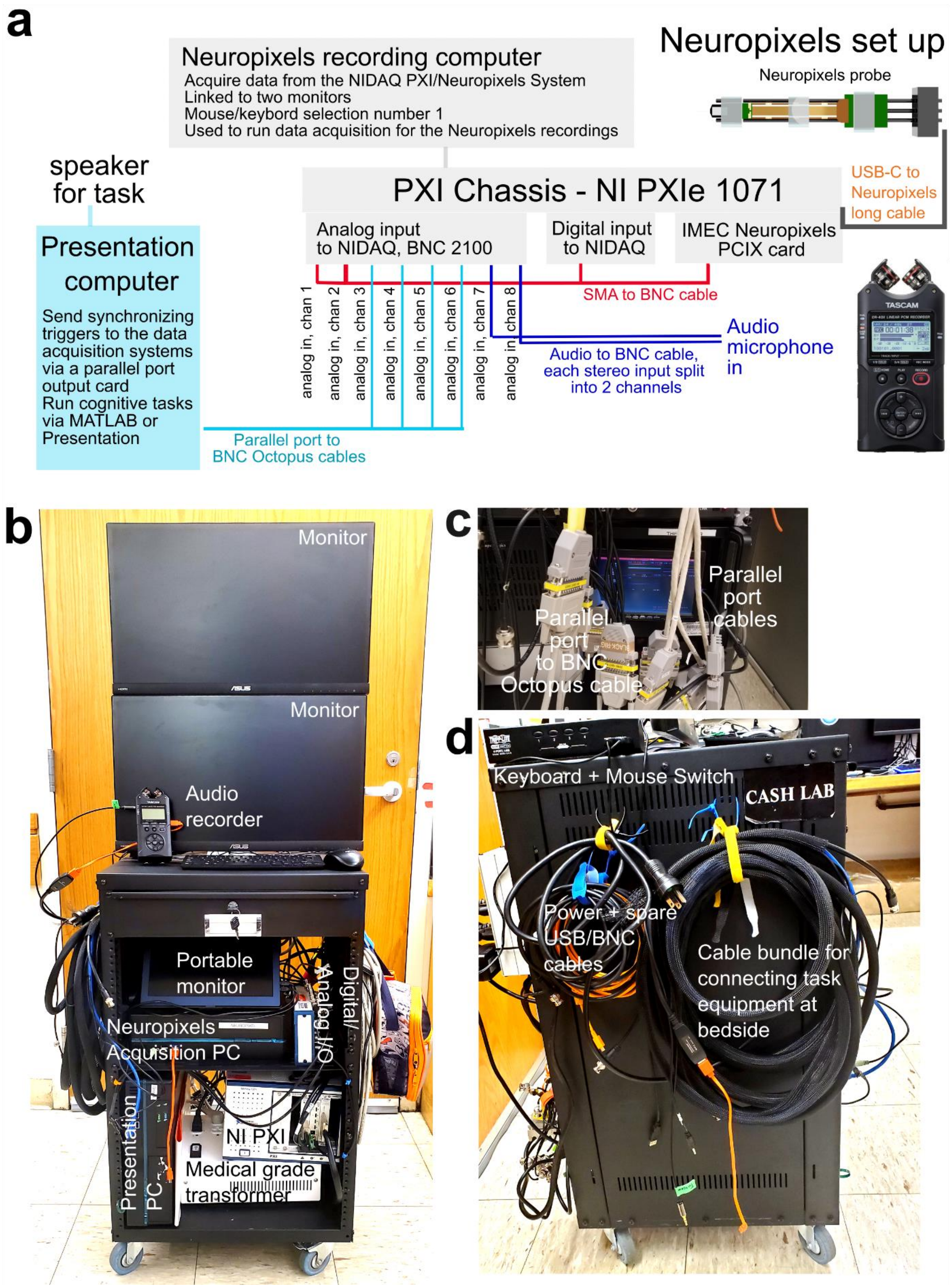
Below are a series of steps used to localize the electrode relative to the brain in the different cases (**Fig. 6**).

1. Electrode localization involves 3D mapping and preoperative and postoperative scans.
2. Following the surgery, the preoperative T1-weighted MRI is used to generate a 3D surface brain map using FreeSurfer scripts [5–11](http://surfer.nmr.mgh.harvard.edu). Images obtained during surgery, locations as indicated using the proprietary software Brainlab (Brainlab, Inc.), and photographs captured during the surgery are aligned to the 3D reconstructions using Blender (v3.0) software (https://www.blender.org/) and MMVT (https://github.com/pelednoam/mmvt) [7,12–15] (**Fig. 6**).
3. For DBS cases, the physical limits imposed by the three-dimensional shape and location of the burr hole result in only certain locations for the insertion of the Neuropixels probe. Therefore, the postoperative CT after the DBS leads were implanted (after the surgery) is overlaid on the preoperative MRI using Mango (http://ric.uthscsa.edu/mango/) [14–16] or FreeView [5,5,10,17] to reconstruct the to-scale burr hole and DBS lead trajectories and map them to the participants’ brains reconstructed using MMVT and FreeSurfer [5–8,10–13].
4. The Neuropixels probe as a to-scale 3D model is then placed in Blender to arrive at the best approximation based on all this information.
5. For open craniotomy cases, the reconstruction involves projecting the surgical image onto the patient’s reconstructed brain using Blender and then placing a 3D model of the Neuropixels probe on that location similar to other coregistration approaches [7,13,17–19] (**Fig. 6**). Angles are calculated from photographs taken during the surgery as well as trajectories limited by the location and angle of the burr hole for DBS surgery

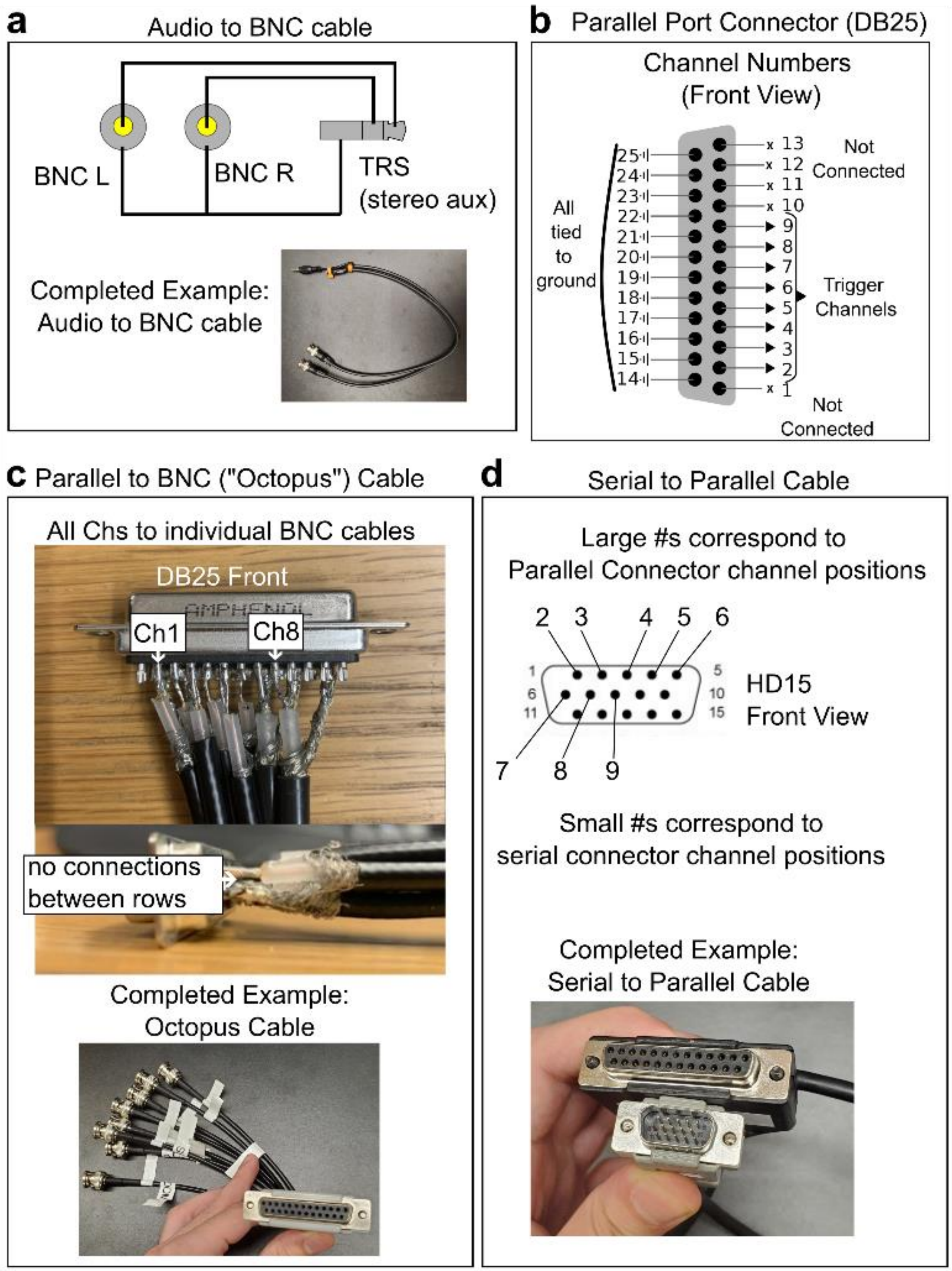
Supplementary Table 1. Data processing parameters per processing step. The steps and parameters below detail the steps taken to produce the deidentified code which is then used for motion registration with DREDge and then interpolation followed by single unit cluster sorting. These parameters are simply some suggestions which can be altered depending on preference and data sets. The code column refers to code and repositories listed in the README file here: https://github.com/Center-For-Neurotechnology/HumanNeuropixelsPipeline

Step	Example code	Input	Output file and/or relevant parameters
Re-saving data for de-identification	/PreprocessingLoading/ExampleFileDeID.m	SpikeGLX .bin files or OpenEphys .dat files with metadata files	Binary file (.bin) saved of the LFP, AP, and DAQ lines of information (SpikeGLX int16 format)
Channel map information from the saved data, OpenEphys	/PreprocessingLoading/ElectrodeLocationsImportSaveOpenEphys.py /PreprocessingLoading/readingChannelPositionsOpenEphysJson.m	OpenEphys metadata files	XXXXXX_ChannelMap.mat file with the x and y coordinates of the recorded Neuropixels channels
Channel map information from the saved data, SpikeGLX	/PreprocessingLoading/SGLXMetaToCoords.m	SpikeGLX metadata files	XXXXXX_ChannelMap.mat file with the x and y coordinates of the recorded Neuropixels channels
Selecting time and channel range to analyze (manual)	RelevantDataExamples/PlottingLFP.m	LFP data, manual entry	Viewing neural data and then manual entry of the time range and channel range to be used in further analyses
Saving LFP data for use with DREDge	/PreprocessingLoading/savingBinaryFilesorMotionRegistraton.m	Binary file (lf.bin) saved of the LFP	All*.lf.bin (LFP files) and channel map (AllinBrain*_ChannelMap.mat) including the recording time range and channel range to use for motion registration
DREDge motion detection	https://github.com/evarol/dredge	All*.lf.bin (LFP files) and channel map (AllinBrain*_Channel	DepthMicronsTracking.csv tracked motion in microns at 250 Hz sample rate

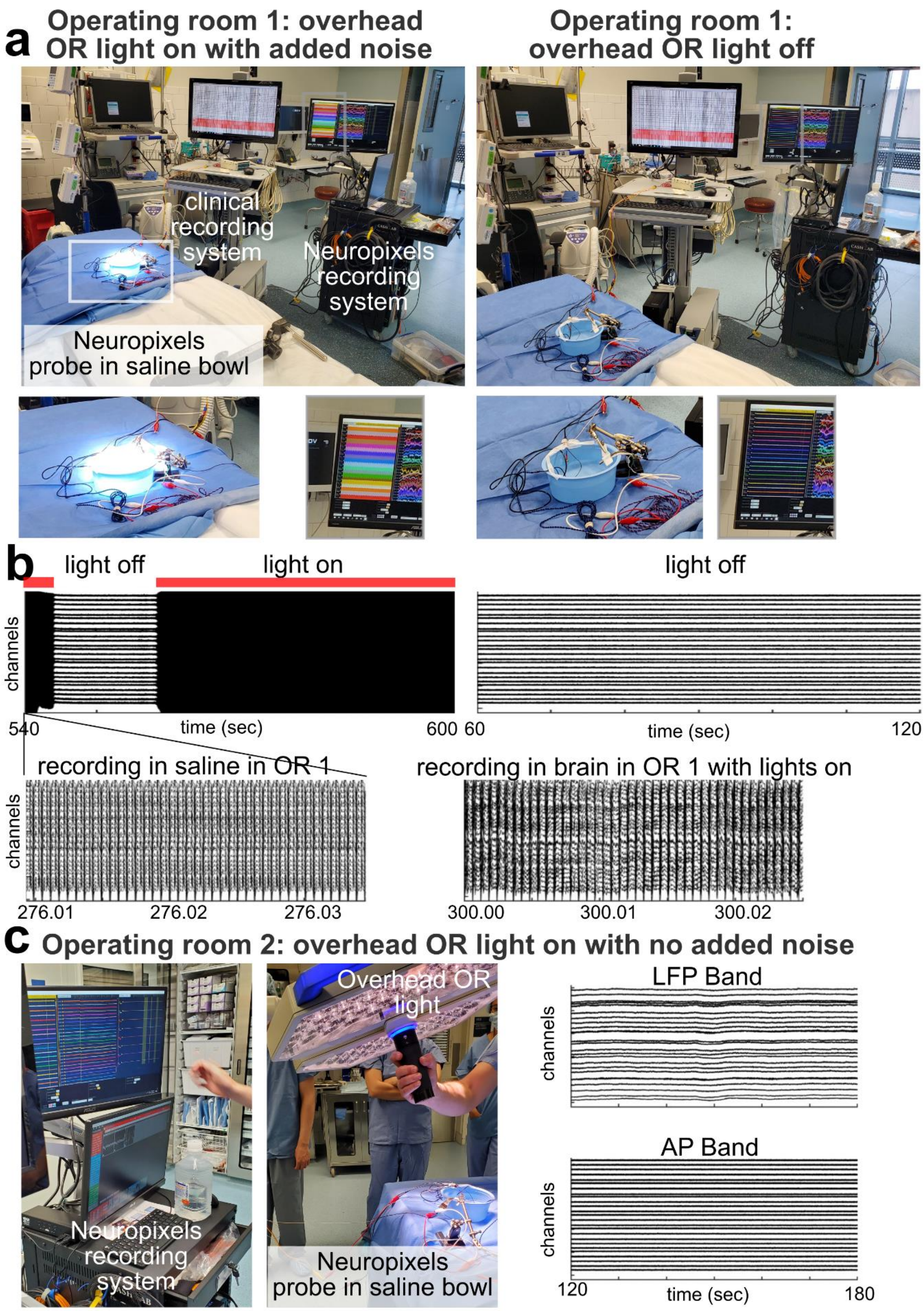
from the LFP	Example Jupyter Notebook:	Map.mat) including the recording time range and channel	
	Parameters (in the code):	Filtered input data: • LFP downsampled to 250 Hz • Spatially filtered across contacts (csd setting) • Band-pass filtered between 0.5 and 250Hz • Analyzed to only include the chosen time range	Setting in DREDge steps: • Rigid online registration • Minimum correlation value between time steps: 0.8 • Maximum displacement allowed = 50 channels • Include a prior in estimating the next step in motion • Adaptive correlation setting turned off
Re-save AP range for interpolation	PruneRecording	Binary file (ap.bin) saved of the AP lines of information (SpikeGLX int16 format) Channel and time range selected	Binary file (ap.bin) saved of the AP lines of information (SpikeGLX int16 format) including the recording time range and channel range to use for motion registration and for further analysis
Interpolate the AP band data	RealignWithDredge_Wrapper Uses Kilosort 2.5 code to perform the interpolation (interpolation currently applied at a rate of BatchSamplesNT = 128 with rate of 30000/BatchSamplesNT	Binary file (ap.bin) saved of the AP lines of information (SpikeGLX int16 format) including the recording time range and channel range to use for motion registration and for further analysis DepthMicronsTracking.csv tracked motion in microns at 250 Hz sample rate	interpolated continuous AP band .dat file adjusted for motion


a
Neuropixels recording computer
Acquire data from the NIDAQ PXI/Neuropixels System
Linked to two monitors
Mouse/keybord selection number 1
Used to run data acquisition for the Neuropixels recordings
Neuropixels set up
Neuropixels probe
speaker for task
PXI Chassis - NI PXIe 1071
USB-C to Neuropixels long cable
Presentation computer
Send synchronizing triggers to the data acquisition systems via a parallel port output card
Run cognitive tasks via MATLAB or Presentation
Analog input to NIDAQ, BNC 2100
Digital input to NIDAQ
IMEC Neuropixels PCIX card
analog in, chan 1
analog in, chan 2
analog in, chan 3
analog in, chan 4
analog in, chan 5
analog in, chan 6
analog in, chan 7
analog in, chan 8
SMA to BNC cable
Audio microphone in
Audio to BNC cable, each stereo input split into 2 channels
Parallel port to BNC Octopus cables
TASCAM
b
Monitor
Monitor
Audio recorder
Portable monitor
Digital/ Analog I/O
Neuropixels Acquisition PC
Presentation PC
NI PXI
Medical grade transformer
c
Parallel port cables
Parallel port to BNC Octopus cable
d
Keyboard + Mouse Switch
CASH LAB
Power + spare USB/BNC cables
Cable bundle for connecting task equipment at bedside

Supplementary Figure 1. Recording system and set up. a. Wiring diagram of the recording system allowing both recording of neural activity with Neuropixels probes and performing cognitive tasks. **b**. Images of the recording system or electrophysiological rig. **c**. Additional cable images allowing for analog channel wiring for synchronization. **d**. Side-view of the set up with additional cabling.

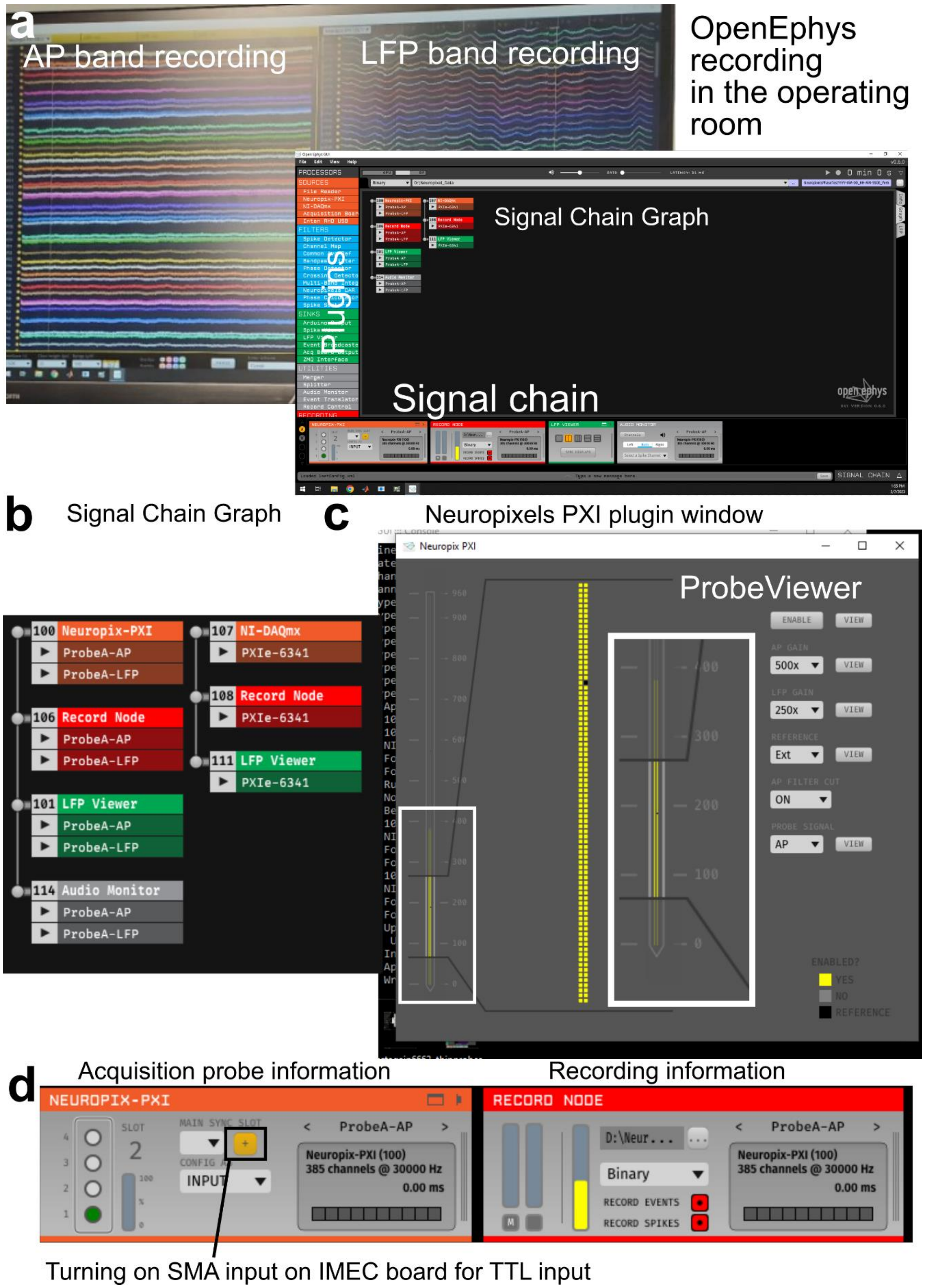


Supplementary Figure 2. Custom cable schematics and examples. a. Simplified schematic showing connections between BNC cable signal wires (gold), shielding (gray) and TRS connector channels in our custom audio to BNC cables used to connect audio devices to I/O modules. **b.** Parallel port connector schematic showing channels used by Presentation PC parallel port output to send TTL signals. Connector positions 2-9 are equivalent to TTL channels 1-8, respectively. **c.** Closeup of soldered parallel to BNC ("octopus") cable connections to illustrate proper signal and ground/shielding connections. **d.** Schematic of serial port connector and corresponding connector positions on parallel port used by presentation PC to send TTLs to the clinical recording system (Natus).

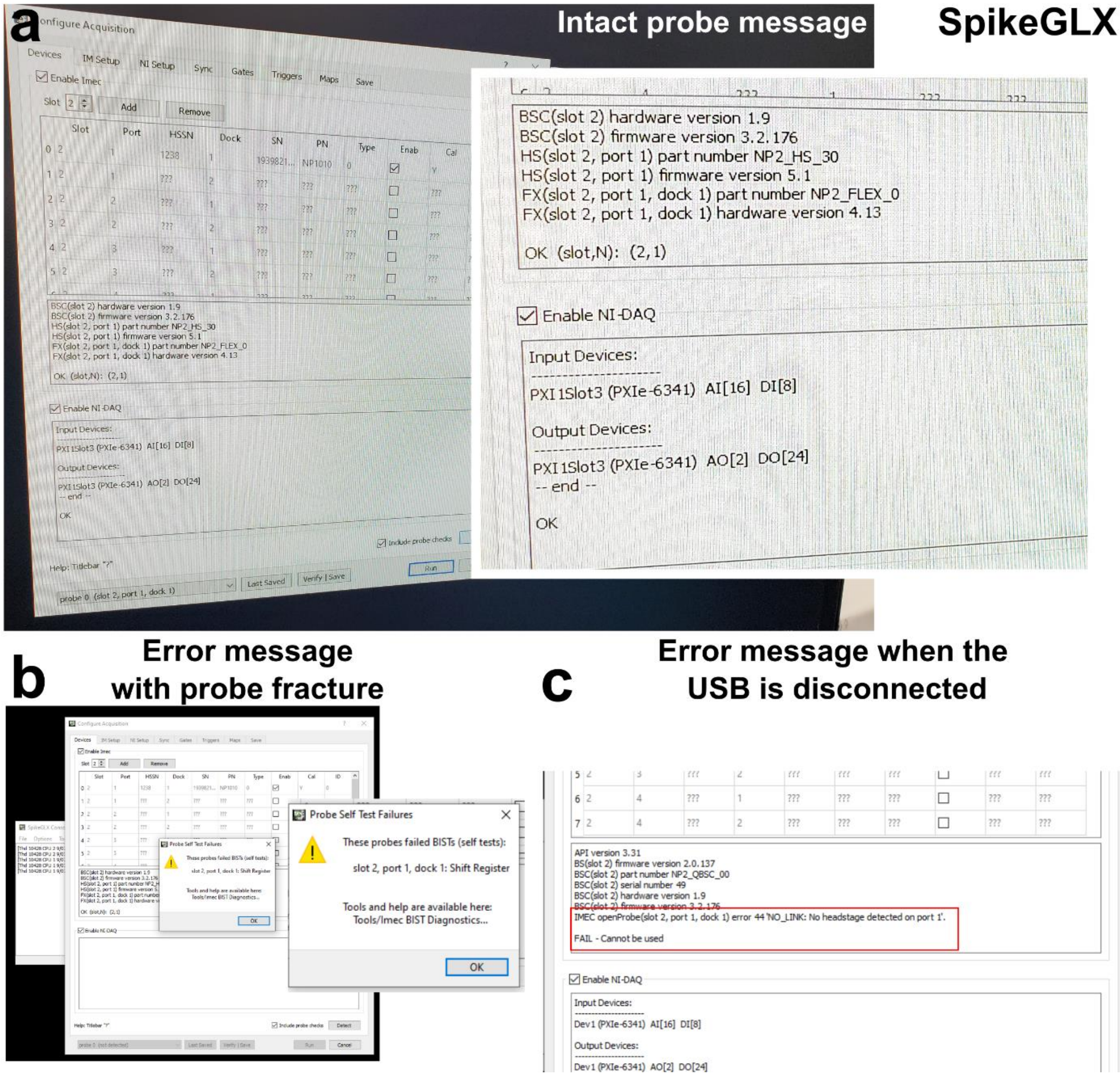


a
Operating room 1: overhead OR light on with added noise
Operating room 1: overhead OR light off
clinical recording system
Neuropixels recording system
Neuropixels probe in saline bowl
b
light off
light on
light off
channels
540
time (sec)
600
60
time (sec)
120
recording in saline in OR 1
recording in brain in OR 1 with lights on
276.01
276.02
276.03
300.00
300.01
300.02
c
Operating room 2: overhead OR light on with no added noise
Neuropixels recording system
Overhead OR light
Neuropixels probe in saline bowl
LFP Band
AP Band
channels
120
time (sec)
180

Supplementary Figure 3. Pre-procedure day noise testing in OR and effect of different overhead light sources on Neuropixels recordings. a. Set up in Operating room 1 without a patient, including the Neuropixels probe in saline with ground and reference, connected to the recording system. Left: with the overhead OR light on with increased noise evident from the recordings (zoomed in below). Right: with the overhead OR light (the moveable large lamp) off. Note the same recordings that are much cleaner. **b.** The recordings in saline (top and bottom left) and in brain with and without the overhead lamp on as indicated by the labels. **c**. Same recording set up in Operating Room 2 with the overhead lamp (OR light) on and directed at the Neuropixels probe. The high frequency noise is not evident in these recordings at the same settings.



Supplementary Figure 4. Open Ephys Software and set up. a. Photographed recording in the operating room including different windows showing the action potential (AP) recording band, the local field potential (LFP) band, and other Open Ephys[4] display features including the signal chain with ongoing recording and synchronization of the recorded signals. **b.** A screenshot of Open Ephys software showing the signal chain used for Neuropixels recordings in the OR as seen in the “graph” tab of the main window. **c.** Screenshot of the Neuropixels PXI window to view the current channel map/reference scheme for the connected probe. Squares in yellow are activated electrode sites. **d.** Screenshots of the Record Node plugin in Open Ephys showing information on the data acquisition as well as the button used to turn ‘on’ the SMA input to record the TTLs from the task.



Supplementary Figure 5 Messages and information using SpikeGLX. **a.** SpikeGLX dialog box readout when an intact probe is connected to the IMEC PXI device and the "Detect" button is selected. **b.** Error popup dialog when a fractured probe is connected and the "Detect" button is selected. **c.** Dialog box output when the USB C cable is detached from the IMEC PXI device.

References:

1. Paulk, A. C. *et al.* Large-scale neural recordings with single neuron resolution using Neuropixels probes in human cortex. *Nature Neuroscience* **25**, 252–263 (2022).
2. Jun, J. J. *et al.* Fully integrated silicon probes for high-density recording of neural activity. *Nature* **551**, 232–236 (2017).
3. Dutta, B. *et al.* The Neuropixels probe: A CMOS based integrated microsystems platform for neuroscience and brain-computer interfaces. *2019 IEEE International Electron Devices Meeting (IEDM)* 10.1.1-10.1.4 (2019) doi:10.1109/IEDM19573.2019.8993611.
4. Siegle, J. H. *et al.* Open Ephys: An open-source, plugin-based platform for multichannel electrophysiology. *Journal of Neural Engineering* **14**, 045003 (2017).
5. Reuter, M., Rosas, H. D. & Fischl, B. Highly Accurate Inverse Consistent Registration: A Robust Approach. *NeuroImage* **53**, 1181–1196 (2010).
6. Reuter, M., Schmansky, N. J., Rosas, H. D. & Fischl, B. Within-Subject Template Estimation for Unbiased Longitudinal Image Analysis. *NeuroImage* **61**, 1402–1418 (2012).
7. Dykstra, A. R. *et al.* Individualized localization and cortical surface-based registration of intracranial electrodes. *NeuroImage* **59**, 3563–3570 (2012).
8. Fischl, B. & Dale, A. M. Measuring the thickness of the human cerebral cortex from magnetic resonance images. *Proceedings of the National Academy of Sciences of the United States of America* **97**, 11050–11055 (2000).
9. Fischl, B., Sereno, M. I. & Dale, A. Cortical Surface-Based Analysis: II: Inflation, Flattening, and a Surface-Based Coordinate System. *NeuroImage* **9**, 195–207 (1999).
10. Fischl, B. *et al.* Sequence-independent segmentation of magnetic resonance images. *NeuroImage* **23**, S69–S84 (2004).
11. Fischl, B. *et al.* Automatically Parcellating the Human Cerebral Cortex. *Cerebral Cortex* **14**, 11–22 (2004).

12. Felsenstein, O. *et al.* Multi-Modal Neuroimaging Analysis and Visualization Tool (MMVT). *arXiV* **1**, 1–29 (2019).

13. Holmes, C. J. *et al.* Enhancement of MR Images Using Registration for Signal Averaging. *Journal of Computer Assisted Tomography* **22**, 324–333 (1998).

14. Lancaster, J. L. *et al.* Automated regional behavioral analysis for human brain images. *Frontiers in Neuroinformatics* **6**, 1–12 (2012).

15. Lancaster, J. L. *et al.* Automated analysis of fundamental features of brain structures. *Neuroinformatics* **9**, 371–380 (2011).

16. Lancaster, J. L. *et al.* Anatomical global spatial normalization. *Neuroinformatics* **8**, 171–182 (2010).

17. Postelnicu, G., Zöllei, L. & Fischl, B. Combined Volumetric and Surface Registration. *IEEE Transactions in Medical Imaging* **28**, 508–522 (2009).

18. Paulk, A. C. *et al.* Microscale Physiological Events on the Human Cortical Surface. *Cerebral Cortex* **31**, 3678–3700 (2021).

19. Yang, J. C. *et al.* Microscale dynamics of electrophysiological markers of epilepsy. *Clinical Neurophysiology* **32**, 2916–2931 (2021).