Integrated analysis of anatomical and electrophysiological human intracranial data

Arjen Stolk1,2*, Sandon Griffin1, Roemer van der Meij3, Callum Dewar1,4, Ignacio Saez1, Jack J. Lin5, Giovanni Piantoni6, Jan-Mathijs Schoffelen2, Robert T. Knight1,7 and Robert Oostenveld2,8

Human intracranial electroencephalography (iEEG) recordings provide data with much greater spatiotemporal precision than is possible from data obtained using scalp EEG, magnetoencephalography (MEG), or functional MRI. Until recently, the fusion of anatomical data (MRI and computed tomography (CT) images) with electrophysiological data and their subsequent analysis have required the use of technologically and conceptually challenging combinations of software. Here, we describe a comprehensive protocol that enables complex raw human iEEG data to be converted into more readily comprehensible illustrative representations. The protocol uses an open-source toolbox for electrophysiological data analysis (FieldTrip). This allows iEEG researchers to build on a continuously growing body of scriptable and reproducible analysis methods that, over the past decade, have been developed and used by a large research community. In this protocol, we describe how to analyze complex iEEG datasets by providing an intuitive and rapid approach that can handle both neuroanatomical information and large electrophysiological datasets. We provide a worked example using an example dataset. We also explain how to automate the protocol and adjust the settings to enable analysis of iEEG datasets with other characteristics. The protocol can be implemented by a graduate student or postdoctoral fellow with minimal MATLAB experience and takes approximately an hour to execute, excluding the automated cortical surface extraction.

Introduction

iEEG enables simultaneous recordings from tens to hundreds of electrodes placed directly on the neocortex (electrocorticography (ECoG)), or intracortically (stereoelectroencephalography (SEEG)). In humans, iEEG is most commonly used in surgery on medication-refractory epilepsy patients because noninvasive techniques such as scalp EEG and MRI do not provide sufficient information to guide the surgery. Each electrode reflects the activity of tens of thousands of neurons1-2, thus, the recording and stimulation of these neural populations facilitates the identification of epileptogenic tissue and the mapping of functional areas of human cortex to guide neurosurgery. The outcome of these procedures can be directly observed when the neural or behavioral response is straightforward, such as speech arrest or muscle movement with direct stimulation3. However, a more complex empirical study requires accurate knowledge of the location of the electrode and the part of the brain from which the local electrophysiological signal has originated. This integrated information is key when undertaking basic and clinical research work aimed at understanding human neural and cognitive processing4,5.

Human iEEG analysis has traditionally relied on stand-alone and ad hoc workflows that separately analyze anatomical and functional aspects of iEEG data. This has meant that research laboratories must assemble software combinations to convert file formats (e.g., “DICOM (Digital Imaging and Communications in Medicine) to NIfTI (Neuroimaging Informatics Technology Initiative)” using MRICovert), co-register anatomical scans (e.g., CT to MRI using SPM6, FSL7, or AFNI8), localize electrodes (e.g., BioImage Suite9), and sort and label electrodes to match the format of the functional recording file (manually or using custom software) in order to process the neuroanatomical data. More efficient workflows for localizing and visualizing electrodes have been developed10-17; however,
Box 1 | Getting started with FieldTrip

**Timing** - 1 min

All the protocol code is directly integrated with and freely available through FieldTrip\(^5\). This MATLAB-based open-source toolbox offers advanced analysis methods for electrophysiological data, such as event-related averaging, frequency and time-frequency analysis, source modeling (for EEG and MEG), connectivity analysis, classification, real-time data processing, and (non)parametric statistical inference. The implementation as a toolbox allows users to perform elaborate and structured analyses of large datasets using the MATLAB command line and batch scripting. Tutorial documentation, answers to frequently asked questions, and example code are available online at [http://www.fieldtriptoolbox.org](http://www.fieldtriptoolbox.org). The toolbox’s infrastructure allows users and developers to relatively easily extend the functionality and implement new algorithms. Over the past decade, the FieldTrip toolbox user base has grown to an estimated 5000 researchers.

To get started with FieldTrip, download the most recent version from its homepage or GitHub, and set up your MATLAB path using the command below:

```matlab
addpath <path to fieldtrip home directory>
```

FieldTrip functionalities, recognizable by an `ft` prefix, typically have a single output argument and one or two input arguments, the first input argument being configuration structure, `cfg`. See some example code below:

```matlab
cfg = {}; cfg.hpfilter = 'yes'; cfg.hpfreq = 1;
data_flt = ft_preprocessing(cfg, data);
```

In this example, input data are processed by `ft_preprocessing` according to the parameters specified in the `cfg` fields, in this case applying a 1-Hz high-pass filter. Each function’s optional parameters are available in the respective function’s header (type `help functionname`) and examples are provided on the wiki.

The `cfg` structure that holds the parameters to the algorithm at the present level is automatically appended to the output data structure, `data_flt.cfg`. Configuration structures used at previous levels are kept in `data_flt.cfg.previous`, `data_flt.cfg.previous.previous`, and so on. This nesting of previous configurations allows for access to the full data provenance at any level of the analysis pipeline (type `help ft_analysispipeline` for how to visualize the pipeline as a flowchart).

This protocol is particularly useful when studying human neural and cognitive processes with iEEG because human iEEG analysis requires the handling of large neuroanatomical and electrophysiological datasets. A wide range of basic and clinical studies use iEEG, including studies of higher-order cognition\(^5,21\) and those involving the localization and understanding of the sources and features of epileptogenic activity\(^2,21,23\). The methodological challenges that iEEG researchers face can be grouped...
into either obstacles that are common to most empirical work or obstacles that are study-specific. This protocol aims to facilitate the resolution of the former while also providing guidance and flexibility that enable study-specific obstacles to be overcome. As an example of the types of data this protocol can be applied to, the protocol we describe here has been used previously to perform group-level investigations of emotion- and language-related neural dynamics and connectivity of the human hippocampus.

Advantages and limitations of the protocol
The main advantages of our protocol are that it (i) guides the researcher from the multitude of raw intracranial data files to integrated observations, in a fast and efficient way; (ii) is directly integrated with a comprehensive and open-source hub for electrophysiological data analysis; (iii) can be readily adapted and automated; (iv) is completely transparent; and (v) produces reproducible workflows and datasets that can be easily shared and generalized to other research modalities. The main limitation is that the MATLAB command-line interface requires some basic programming knowledge, which may require more initial learning as compared with the execution of computer commands through a (black box) graphical user interface. However, the use of computer commands can be relatively easily mastered by following this protocol, enabling the use of batch scripting to efficiently deal with repeated analyses within and across subjects. In addition, using computer commands ultimately enables a deeper understanding of the underlying algorithms.

Human intracranial datasets come in different sizes and shapes, and can be processed for different purposes, making it important for a protocol to strike the right balance between efficiency and flexibility. This need is further amplified by the relatively unique nature of intracranial data, typically imposing greater demands on alternative options and strategies in the analysis than noninvasive data recorded with more standardized hardware and software in dedicated laboratory settings. In addition to providing a quick guide to interpretable results, our protocol allows easy switching between analysis methods to accommodate the processing of data from different cases and situations. By changing a single parameter at execution, one can, for instance, readily apply a different fusion cost function or filter setting. The well-defined data structures created by the protocol contain full processing details at any level of the analysis pipeline, enabling researchers to adapt to circumstances by selecting between the processing algorithms in a transparent and reproducible way.

The spatiotemporal precision of iEEG provides a unique window on neural processing. The size and dimensions of this window, however, may grow disproportionately large with certain types of analyses, complicating the overall interpretability of the data. Starting from the two dimensions of the raw neural signal (channels and time), a time–frequency analysis, for instance, results in three dimensions in the output (power as a function of channel, time, and frequency), whereas between-channel connectivity analysis expands the combinatorial space to four dimensions. Our protocol enables the interactive manipulation of anatomically informed graphical representations of the neural data, which facilitates visualization of the multidimensional outcome of an iEEG analysis.

Integration with FieldTrip
In addition to the complete transparency that comes with an open-source toolbox, the integration with FieldTrip enables iEEG researchers to build on algorithms for reading raw data in various formats, data preprocessing, event-related potential analysis, spectral analysis, source modeling, connectivity analysis, classification, real-time data processing, and statistical inference. When applied to human iEEG data, these methods allow characterization of neural information flow with a level of detail inaccessible to noninvasive techniques. In addition, invasive and noninvasive human electrophysiology can be visualized together using very similar analysis pipelines to enable an integrated presentation of neural processing or a comparison of MEG/EEG source reconstruction methods with iEEG.

The open-source development model allows for additional components to be relatively easily added to the protocol. For example, several techniques exist to compensate for electrode displacement due to the ‘brain-shift’ phenomenon explained below. Each of these techniques has different strengths and weaknesses; thus, the best technique to use can be determined on a case-by-case basis. FieldTrip’s modular architecture enables developers to incorporate new techniques and users to subsequently utilize these techniques by changing a single parameter at function call. This facilitates the use of the protocol in new research areas, including single- and multi-unit recordings, ‘NeuroGrid’ recordings, wireless ‘Neural Dust’ recordings, (deep) brain stimulation, and...
multimodal imaging\textsuperscript{12}. We plan to coordinate with these new electrophysiological endeavors and to continue sharing analysis code with other software packages.

**Compatibility with FreeSurfer**

The protocol is compatible with the freely available FreeSurfer software package\textsuperscript{37}. Although optional, processing of the anatomical MRI with FreeSurfer (Step 6) offers several advantages for subsequent analysis and data interpretation. Processing the MRI with FreeSurfer results in the creation of a cortical mesh, consisting of approximately equally sized triangles that form a topological sphere for each of the cerebral hemispheres. This cortical mesh enables an anatomically realistic representation of the electrophysiological data on the neocortex (e.g., bottom center in Fig. 1). A smoothed version of the extracted cortical surface can be used to compensate for electrode displacement due to brain shift (Step 23). Moreover, FreeSurfer automatically registers the subject’s brain to a template brain on the basis of its cortical gyration pattern, an aspect of brain structure that remains difficult to accurately normalize using volume-based registration techniques due to its complexity and variability across subjects\textsuperscript{38,39}. Our protocol uses the resulting surface-registration maps to link surface electrode positions to their template homologs, preserving the spatial relationship between cortical folds and electrodes in each subject (Step 30). This makes the surface-based registration technique particularly effective for generalizing across subjects, facilitating more accurate group-level analysis based on individual peak activity sites or cortically interpolated activity (i.e., projecting and averaging activity from multiple subjects on a cortical mesh of FreeSurfer’s ‘fsaverage’ template brain instead of the subject’s brain as in Step 51). Finally, FreeSurfer-generated atlases are convenient for representations of neural and anatomical data for a single subject (Step 53), as they are defined in native subject space. Other supported atlases are defined in standardized (e.g., Montreal Neurological Institute (MNI)) space and require the added step of transforming electrode positions to that space.

**Specific considerations when processing human intracranial data**

Anatomical images, typically MRI and CT scans, are used as part of the epilepsy diagnostic and surgical procedures. A pre-implant MRI shows the anatomy of the head, including the brain, and is used to identify structural abnormalities. An MRI is also instrumental in guiding SEEG electrode implantation subsequent to the clinical decision to record intracranially. A post-implant CT shows high-intensity objects such as the electrodes and skull but lacks details of brain anatomy. To obtain knowledge of an electrode’s location in relation to the brain’s anatomy, the two scans must be fused.

After fusion of the pre- and postoperative anatomical images, electrodes that have been surgically placed on the cortical surface occasionally appear ‘buried’ within the cortical tissue, sometimes more than a centimeter deep\textsuperscript{40–45}. This electrode displacement is typically due to ‘brain shift’, the inward sinking of the brain post implant that is most commonly observed with electrocorticographic surface grid electrodes. The brain shift reflects tissue displacement caused by the electrodes themselves and by subdural fluid loss or accumulation. As noted, the displacement is most pronounced directly below a craniotomy and is usually minimal for implants solely involving burr holes\textsuperscript{45}. It is important to account for this brain shift in order to accurately align electrode-specific signals with the local cortical anatomy. Several labs have developed re-alignment techniques to compensate for electrode displacement due to brain shift, reducing localization error to <3 mm as compared with that of intraoperative photographs\textsuperscript{11,25–32}. Our protocol currently supports two of these techniques to project electrode grids back to the cortical surface. One method that we demonstrate in Step 23 involves an optimization algorithm that minimizes an energy function defined by inter-electrode distances and global deformation of the electrode configuration\textsuperscript{25}. The other method projects electrodes to the surface of the brain in the direction of the local norm vector of the electrode grid\textsuperscript{25}.

Electrode localization can also be performed using post-implant MRIs, although these are not commonly acquired in a clinical setting. These scans show the brain anatomy after electrode implantation, so brain shift is not an issue. In a T1-weighted MRI, electrodes appear dark, owing to the magnetic susceptibility artifact. This is generally not an issue for recordings with depth electrodes (SEEG), in which the electrodes are visible as dark voids in the higher-intensity brain tissue. Electrode grids and strips (ECoG), on the other hand, are placed directly on the cortical surface. This complicates their identification, as the electrodes are surrounded by cerebral spinal fluid, which also appears dark on a T1 scan (but see refs \textsuperscript{27,46–48} for workarounds). In the rare case that a post-implant MRI is available that is of sufficient quality to clearly show the electrodes, the CT preprocessing and fusion steps (Steps 9–15) may be left out. In this situation, the electrode localization may be
performed on the post-implant MRI. Furthermore, in the case of single-unit recordings, a post-implant MRI may be required to localize the microwires. However, if the post-implant MRI is of unsatisfactory quality in regard to brain anatomy, for instance, due to electrode-induced MR signal distortion, we recommend fusing the post-implant MRI with the pre-implant MRI, as if it were a post-implant CT.

Neural recordings are typically part of the ongoing clinical monitoring and come in various file formats. Each data channel represents, as a function of time, the electric potential difference, obtained
with either a bipolar or referential electrode scheme. Thus, the electrodes are pairwise linked or referenced to a single, common electrode during acquisition. The latter montage has the benefit that the recordings can be easily remontaged to a more preferred scheme in the off-line analysis\(^5\). The markers or triggers for stimulus-onset times and responses are typically recorded simultaneously in a dedicated channel, allowing for precise synchronization of experimental scenarios with the neural recording.

**Overview of the procedure**

The protocol has two parallel but interrelated workflows, as shown in Fig. 1. Anatomical data are processed in the first workflow. The main components of the first workflow are the preprocessing and fusion of the anatomical images, and electrode placement (Steps 1–20). Secondary activities that might be required include cortical surface extraction with FreeSurfer (Step 6), and brain-shift compensation, spatial normalization, and anatomical labeling (Steps 21–34). Generally, the anatomical workflow aims to obtain estimates of the electrode locations in relation to the individual and atlas-based brain anatomy, which is a one-time procedure for each subject. The second workflow improves the signal-to-noise ratio and extracts the relevant features from the electrophysiological data while preparing for subsequent analyses. The minimal component is the preprocessing of the neural recordings (Steps 35–44), but this part of the protocol can also include follow-up activities such as time–frequency (Steps 45 and 46) and single-subject or group-level statistical analysis (not covered in the procedure). Generally, the specifics of the functional workflow depend ultimately on the clinical or research question at hand and contingencies in the experimental paradigm.

The two workflows become connected for the first time during the electrode-placement activity (Step 17), which offers the opportunity to directly link anatomical locations to electrode labels corresponding to the neural recordings. Electrode placement involves a graphical user interface designed for efficient yet precise identification of electrodes in even the most challenging cases. The integration of the two workflows culminates in an interactive and anatomically informed data-exploration tool and the ability to represent functional and epileptiform neural activity overlaid on cortical and subcortical surface models, in figure or video format (Steps 47–57).

**Implementation and adaptation of the procedure**

All parts of the procedure, except for FreeSurfer, run on a single universal platform (MATLAB), which enables relatively easily automated procedures that can deal with repeated analyses within and across subjects. We recommend that the user construct a single script for a single subject by copying and pasting code from this protocol into the MATLAB editor (e.g., Supplementary Data 1), and evaluating segments of that script in the MATLAB command window. Once the script produces satisfactory results, it can be converted into a batch analysis by breaking it into separate components. By looping around the separate components for all subjects, the entire analysis pipeline for all subjects in a study can easily be executed and intermediate results can be saved and evaluated.

The whole batch can be documented and shared, or re-evaluated with different parameter settings, as appropriate. Because single parameters can be changed at a function call, alternation between various fusion, localization, projection, normalization, filtering, remontaging, and spectral estimation algorithms can be achieved, accommodating analysis of different cases and situations. The output data structures are kept consistent across the different algorithms, and the parameters of the used algorithm are appended to allow for access to the full data provenance at any level of the analysis pipeline (Box 1).

**Datasets that can be processed**

**Example dataset**

In the procedure, we explain each part of the data processing but also provide specific instructions that enable analysis of an example iEEG dataset. Further information about this dataset is provided in the Materials section.

We chose this iEEG dataset as an illustrative example for three reasons. First, it contains neural recordings from both cortical grid (ECoG) and stereotactically inserted depth electrodes (SEEG), requiring strategies for dealing with each type, as well as their combination, in the analysis. Second, the pre-implant MRI is not of the best quality (a contrast agent was used), electrodes of adjacent cortical grids have seemingly merged with one another in the post-implant CT, and there is substantial electrode displacement owing to a subdural hygroma contributing to brain shift. These issues are common in intracranial data analysis and, hence, it is important that users understand how to deal
with nonideal data. By processing this example dataset, users must undertake all the fundamental steps required to analyze intracranial data, and these steps are independent of the experimental paradigm being investigated\(^{20,24,50}\). This example demonstrates the analysis of task-related high-frequency-band activity (\(\sim 70-150\) Hz), a prominent neural signature in intracranial data that has been associated with neuron population-level firing rate\(^{5,51-54}\). Many other examples of analyses that can be undertaken, such as event-related potential analysis, connectivity analysis, and statistical analysis, have been described in detail elsewhere\(^{55-57}\).

### Supported data formats

The following anatomical data formats can be analyzed using this protocol:

- AFNI (*head, *brik)
- Analyze (*img, *.hdr)
- ANT (*.mri)
- Biolmage Suite (*mgrid)
- DICOM (*.dcm, *.ima)
- FreeSurfer (*.mgz, *.mgh)
- MINC (*.mnc)
- NITI (*.nii, *.nii.gz)

The following electrophysiological data formats can be analyzed using this protocol:

- Anywave (*.ah5)
- BCI2000 (*.dat)
- BESA (*.besa)
- Blackrock (*.nev, *.ns#)
- Cambridge Electronic Design (*.smr)
- European Data Format (*.edf)
- GTec (*.mat, *.hdf5)
- Micromed (*.trc)
- Neuralynx (*.ncs, *.nse, *.nts, *.nst, *.ntt, *.nev)
- Neuromag (*.fif)
- Neuroscope (*.eeg, *.dat, *.xml)
- Nihon Kohden (*.m00, *.eeg)
- Plexon (*.ddt, *.nex, *.plx)
- Various EEG, MEG, NIRS, and eye-tracker data formats

### Materials

#### Equipment

- Suitable datasets that can be used are discussed in the Introduction, and details of the example dataset are given in Box 2. **CAUTION** The acquisition and subsequent analysis of data from human subjects must conform to institutional and national regulations. Informed consent must be obtained from experimental subjects for all procedures being undertaken.

#### Software

- MATLAB environment (MathWorks; installation and licensing through [http://www.mathworks.com](http://www.mathworks.com))
- FieldTrip toolbox (Box 1; freely available at [http://www.fieldtriptoolbox.org](http://www.fieldtriptoolbox.org), code: [https://github.com/fieldtrip/fieldtrip](https://github.com/fieldtrip/fieldtrip))
- FreeSurfer software suite for cortical surface extraction (optional; freely available at [http://www.freesurfer.net](http://www.freesurfer.net))

### Procedure

#### Specification of subject ID  

**Timing -1 min**

1. Specify the subject ID using the below command.

   ```matlab
   subjID = 'SubjectUC129'
   ```

   This ID will be used in the file naming, in addition to information about the type of data (e.g., MRI, CT), the coordinate system that is used (e.g., ACPC, MNI), and the
process(es) that were applied to it (e.g., ‘f’ for fusion). For example, a CT scan that is aligned to the ACPC (Anterior Commissure - Posterior Commissure) coordinate system and that has just been fused with the anatomical MRI is written out to a file as subjID_CT_acpc_f.nii.

Box 2 | Example dataset

We describe specifically how to process an example dataset acquired at the Medical Center of the University of California, Irvine. The Office for the Protection of Human Subjects of the University of California, Berkeley, approved the study, and the subject gave informed consent. The components of the dataset are listed below. The dataset contains three anatomical images plus neural recordings from 96 ECoG and 56 SEEG electrodes that were implanted as part of preparation for epilepsy surgery. The neural data were recorded in the context of an experiment that required the patient to press a button with the right hand when hearing a target tone. All neural recordings were acquired using a Nihon Kohden recording system with a JE-120A amplifier, analog-filtered above 0.01 Hz and digitally sampled at 5 kHz. The sequence imaging parameters used for the MRI were MP-RAGE, 256 × 256 × 176 mm with 1 × 1 × 1-mm isotropic voxels. The original dataset (after defacing the imaging data with ft_defacevolume) and the processed results are available for download from the online repository at https://doi.org/10.5281/zenodo.1201560. Raw DICOM images and recording files are not shared, in order to protect the subject’s identity.

Anatomical images
- Pre-implant T1-weighted MRI, SubjectUCI29_MRI_acpc.nii (MRI, Siemens 3T TrioTim)
- Post-implant CT, SubjectUCI29_CT_acpc_f.nii (CT, Philips iCT 256)
- Post-implant T1-weighted MRI, SubjectUCI29_postimpl_MR_acpc_f.nii (MRI, Siemens 1.5T Avanto). This scan is not used in the Procedure but is nevertheless included for completeness.

Neural recordings
- The preprocessed neural recordings are in SubjectUCI29_data.mat.
- 64-Contact cortical grid with left parietal coverage (Integra, 4×8 layout, 10-mm inter-electrode spacing, labels have an ‘LTG’ prefix)
- Eight-contact linear depth electrode targeting the left amygdala (Ad-Tech, 5-mm inter-electrode spacing, labels have a ‘LAM’ prefix)
- Eight-contact linear depth electrode targeting the left hippocampus head (Ad-Tech, 5-mm inter-electrode spacing, labels have an ‘LHH’ prefix)
- Eight-contact linear depth electrode targeting the right hippocampus tail (Ad-Tech, 5-mm inter-electrode spacing, labels have an ‘LTH’ prefix)
- Eight-contact linear depth electrode targeting the right amygdala (Ad-Tech, 5-mm inter-electrode spacing, labels have a ‘RAM’ prefix)
- Eight-contact linear depth electrode targeting the right hippocampus head (Ad-Tech, 5-mm inter-electrode spacing, labels have an ‘RHH’ prefix)
- Eight-contact linear depth electrode targeting the right hippocampus tail (Ad-Tech, 5-mm inter-electrode spacing, labels have an ‘RHT’ prefix)
- Eight-contact linear depth electrode targeting the right occipital cortex (Ad-Tech, 5-mm inter-electrode spacing, labels have a ‘ROC’ prefix)

Box 3 | Coordinate system determination ● Timing - 1 min

Coordinate systems define the orientation and units of the x, y, and z axes of an anatomical volume, in addition to an origin point along the brain’s midline (e.g., AC). Here, we provide a guideline for determining the native coordinate system of the MRI and CT scans and, in particular, whether they have a left-to-right or a right-to-left orientation. Knowledge of the orientation of the left-right axis of the scan’s native coordinate system provides the necessary context for demarcating the right-hemisphere landmark in the succeeding alignment step. Although the interpretation of posterior-anterior and inferior-superior axes is straightforward from axial, coronal, or sagittal slices of the brain, differentiating left from right requires a 3D context. To accomplish this, we recommend using ft_determine_coordsys, which depicts an anatomical volume as three intersecting, orthogonal slices and labels the x, y, and z axes. This allows the axis that represents the left-right axis to be identified and, importantly, determines whether that axis has a left-to-right or a right-to-left orientation (see Supplementary Video 1 for further guidance).

1 Visualize the coordinate system of the MRI or CT by inputting the following command:

   ft_determine_coordsys(mri)

2 Determine which of the three axes, x, y, or z, runs through or along the left-right axis of the subject’s head. This axis is the left-right axis for this anatomical volume.

3 Determine the orientation of the left-right axis. If the values on the left-right axis increase to the right (indicated by a ‘+’ sign), then the scan has a left-to-right orientation. If the values on the left-right axis increase to the left, then the scan has a right-to-left orientation.

4 Write down the orientation of the scan’s left-right axis.
Preprocessing of the anatomical MRI ● Timing ~2 min

2 Import the anatomical MRI (for the example dataset, this is the file named SubjectUCI29_MR_acpc.nii) into the MATLAB workspace using ft_read_mri with the below command.

```
mri = ft_read_mri(<path to MRI file>);
```

The MRI comes in the format of a single file with an .img or .nii extension, or a folder containing a series of files with a .dcm or .ima extension (The code in Supplementary Data 2 may aid in the search and visualization of these DICOM series.).

3 Determine the native orientation of the anatomical MRI's left–right axis using ft_determine_coordsys as described in Box 3 and Supplementary Video 1.

4 Using the below command, align the anatomical MRI to the ACPC coordinate system, a preferred convention for the FreeSurfer operation optionally used at Step 6. In this coordinate system, the origin (coordinate \([0,0,0]\)) is at the anterior commissure (AC), the \(y\) axis runs along the line between the AC and the posterior commissure (PC), and the \(z\) axis lies in the midline dividing the two cerebral hemispheres. Specify the AC and PC, an interhemispheric location along the midline at the top of the brain, and a location in the brain’s right hemisphere. If the scan was found to have a left-to-right orientation in the previous step, the right hemisphere is identified as the hemisphere having larger values along the left–right axis. Vice versa, in a right-to-left system, the right hemisphere has smaller values along that axis than does its left counterpart (see Supplementary Video 2 for further guidance).

```
cfg = [];
cfg.method = 'interactive';
cfgcoordsys = 'acpc';
mri_acpc = ft_volumerealign(cfg, mri);
```

▲ CRITICAL STEP To correctly fuse the MRI and CT scans at a later step, accuracy in demarcating the right-hemisphere landmark is important for avoiding an otherwise hard-to-detect flip of the scan’s left and right orientation.

5 Write the preprocessed anatomical MRI out to a file as shown below.

```
cfg = [];
cfg.filename = [subjID '_MR_acpc'];
cfg.filetype = 'nifti';
cfg.parameter = 'anatomy';
ft_volumewrite(cfg, mri_acpc);
```

(Optional) Cortical surface extraction with FreeSurfer ● Timing ~10 h, automatic

▲ CRITICAL Although optional, processing of the anatomical MRI with FreeSurfer can be beneficial for subsequent analysis and data interpretation, as discussed in the Introduction.

6 Execute FreeSurfer's recon-all functionality from the Linux or MacOS terminal (Windows via VirtualBox), or from the MATLAB command window as detailed below.

```
fshome = <path to freesurfer home directory>;
subdir = <path to subject directory>;
mrfi le = <path to subject MR_acpc.nii>;
system(['export FREESURFER_HOME=' fshome ' ; ' ... 'source $FREESURFER_HOME/SetUpFreeSurfer.sh; ' ... 'mri_convert -c -oc 0 0 0 mrfi le ' [subdir '/tmp.nii'] ' ; ' ... 'recon-all -i ' [subdir '/tmp.nii'] ' -s ' 'freesurfer' '-sd ' subdir ' -all'])
```

This set of commands will create a folder named ‘freesurfer’ in the subject directory, with subdirectories containing a multitude of FreeSurfer-generated files.

▲ PAUSE POINT FreeSurfer’s fully automated segmentation and cortical extraction of the anatomical MRI can take 10 h or more, so the program can be left to run overnight. To avoid delay when working through the example dataset, the online repository containing the example dataset also contains the output from FreeSurfer that is obtained from this step in a folder named ‘freesurfer’.

7 Import the extracted cortical surfaces into the MATLAB workspace and examine their quality.

```
pial_lh = ft_read_headshape(<path to freesurfer/surf/lh.pial>);
pial_lh.coordsys = 'acpc';
ft_plot_mesh(pial_lh);
lighting gouraud;
```
Repeat the above code using `rh.pial` to visualize the pial surface of the right hemisphere.

**TROUBLESHOOTING**

8 Import the FreeSurfer-processed MRI (from Step 6) into the MATLAB workspace for the purpose of fusing with the CT scan at Step 13, and specify the coordinate system to which it was aligned in Step 4, using the following command:

```matlab
fsmri_acpc = ft_read_mri(<path to freesurfer/mri/T1.mgz>);
fsmri_acpc.coordsys = 'acpc';
```

**Preprocessing of the anatomical CT**

9 Import the anatomical CT (for the example dataset, this is the file named ‘SubjectUCI29_CT_acpc.nii’) into the MATLAB workspace using the below command.

```matlab
ct = ft_read_mri(<path to CT file>);
```

Similar to the MRI, the CT scan comes in the format of a single file with an .img or .nii extension, or a folder containing a series of files with a .dcm or .ima extension. (The code in Supplementary Data 2 can be used to search and visualize a DICOM series.)

10 If the laterality of the electrode implantation is not known, determine the native orientation of the anatomical CT’s left–right axis using `ft_determine_coordsys` and following the steps given in Box 3 and Supplementary Video 1.

11 Align the anatomical CT to the CTF head surface coordinate system by specifying the nasion (at the root of the nose), left and right pre-auricular points (just in front of the ear canals), and an interhemispheric location along the midline at the top of the brain using the following command (this step is also shown in Supplementary Video 3):

```matlab
cfg = [];
cfg.method = 'interactive';
cfg.coordsys = 'ctf';
ct_ctf = ft_volumerealign(cfg, ct);
```

The CT scan is initially aligned to the CTF head surface coordinate system because the ACPC coordinate system used for the MRI relies on neuroanatomical landmarks that are not visible in the CT.

▲ CRITICAL STEP To correctly fuse the MRI and CT scans at Step 13, accuracy in demarcating the right and left pre-auricular landmark in this step is important for avoiding an otherwise hard-to-detect flip of the scan’s left and right orientation.

12 Automatically convert the CT’s coordinate system into an approximation of the ACPC coordinate system, the same system to which the anatomical MRI was aligned, using the below command.

```matlab
ct_acpc = ft_convert_coordsys(ct_ctf, 'acpc');
```

**Fusion of the CT with the MRI**

13 Fuse the CT with the MRI using the below command.

```matlab
cfg = [];
cfg.method = 'spm';
cfg.spmversion = 'spm12';
cfg.coordsys = 'acpc';
cfg.viewresult = 'yes';
ct_acpc_f = ft_volumerealign(cfg, ct_acpc, fsmri_acpc);
```

Fusion of the scans is necessary to link the electrode locations in the anatomical CT to their corresponding locations in the anatomical MRI. Given that both scans are from the same subject and their common denominator is the skull, a rigid-body transformation suffices for their alignment under normal circumstances (the default technique when using the SPM method in FieldTrip).

14 Carefully examine the interactive figure that is produced after the co-registration is completed, showing the MRI and fused CT superimposed. A successful fusion will show tight interlocking of CT-positive skull (in blue) and MRI-positive brain and skin tissue (in red).

▲ CRITICAL STEP Accuracy of the fusion operation is important for obtaining precise knowledge of the location of the electrode and the part of the brain from which the local electrophysiological signal has originated. It is also important for correctly placing the electrodes in an anatomical context at Step 33.

**TROUBLESHOOTING**
15 Write the MRI-fused anatomical CT out to a file using the command below.

cfg = [];  
cfg.filename = [subjID '_CT_acpc_f'];  
cfg.filetype = 'nifti';  
cfg.parameter = 'anatomy';  
ft_volumewrite(cfg, ct_acpc_f);

Electrode placement ● Timing -15 min

16 Import the header information from the recording file, if possible, using the command below.

hdr = ft_read_header(<path to recording file>);

By giving the electrode labels originating from the header as input to ft_electrodeplacement in the next step, the labels will appear as a to-do list during the interactive electrode-placement activity. A second benefit to this step is that the electrode locations can be directly assigned to labels collected from the recording file, obviating the need to sort and rename electrodes to match the electrophysiological data.

17 Localize the electrodes in the post-implant CT with ft_electrodeplacement, as shown in Fig. 2. Clicking an electrode label in the list will directly assign that label to the current crosshair position in the CT scan. Several features facilitate precise navigation of the anatomical CT, such as a zoom mode, a magnet option that transports the crosshair to the nearest weighted maximum (or minimum in the case of a post-implant MRI), and an interactive 3D scatter figure that is linked to the 2D volume representations. Use the command below to pass on the pre-implant MRI, fsmri_acpc, to ft_electrodeplacement to allow toggling between the CT and MRI views for the identification of specific electrodes based on their anatomical location. Generally, electrode 1 is the electrode farthest away from the craniotomy or burr hole in the case of depths and single-row strips. To determine the numbering of grid and multirow strip electrodes, it is important that careful notes be taken during surgery and recording.

cfg = [];  
cfg.channel = hdr.label;  
elec_acpc_f = ft_electrodeplacement(cfg, ct_acpc_f, fsmri_acpc);

TRoubleshooting

18 Examine whether the variables in the resulting electrode structure elec_acpc_f match the recording parameters, e.g., the number of channels stored in the label field, by typing elec_acpc_f into the command window.

elec_acpc_f =  
unit: 'mm'  
coordsys: 'acpc'  
label: {152x1 cell}
The electrode and channel positions are stored in the `elecpos` and `chanpos` fields, respectively. The `elecpos` field contains the original electrode positions. With the exception of possible brain-shift compensation, this field is not adjusted. The channel positions in the `chanpos` field are initially identical to the electrode positions but may be updated to accommodate off-line adjustments in channel combinations, i.e., during remontaging. For bipolar EEG data, the channel position considered the best is between the two corresponding electrode positions. The `tra` field is a matrix with the weight of each electrode into each channel, which at this stage merely is an identity matrix reflecting one-to-one mappings between electrodes and channels.

Using the command below, visualize the MRI along with the electrodes and their labels and examine whether they show the expected behavior.

```matlab
ft_plot_ortho(fsmri_acpc.anatomy, 'transform', fsmri_acpc.transform, 'style', 'intersect');
ft_plot_sens(elec_acpc_f, 'label', 'on', 'fontcolor', 'w');
```

**Troubleshooting**

Using the command below, save the resulting electrode information to a file.

```matlab
save([subjID '_elec_acpc_f.mat'], 'elec_acpc_f');
```

### Brain-shift compensation (optional for cortical grids and strips)

In the case of 'brain shift', i.e., the inward displacement of brain tissue and electrodes due to pressure changes related to the craniotomy, re-alignment of electrode grids to the pre-implant cortical surface may be necessary. To prevent inwardly displaced electrodes from being incorrectly placed in nearby cortical sulci during back-projection, create a smooth hull around the cortical mesh generated by FreeSurfer\(^6\) using the command below. This hull tracks the (exposed) outer surface on which the cortical grid rested.

```matlab
cfg = [];
cfg.method = 'cortexhull';
cfg.headshape = <path to freesurfer/surf/lh.pial>;
cfg.fshome = <path to freesurfer home directory>;
hull_lh = ft_prepare_mesh(cfg);
```

Save the hull to a file using the command below.

```matlab
save([subjID '_hull_lh.mat'], hull_lh);
```

Using the command below, project the electrode grids to the surface hull of the implanted hemisphere. Given that different grids can move independently from one another and that the projection algorithm specified in `cfg.warp` considers the global electrode configuration of a grid\(^3\), it is recommended to re-align electrode grids individually by running separate re-alignment procedures for each grid. Here, we re-align the electrodes of the left parietal grid, followed by alignment of the electrodes of the left temporal grid (LPG and LTG, respectively) and store the updated grid electrode information in a new variable together with the unaltered coordinates of the depth electrodes.

```matlab
elec_acpc_fr = elec_acpc_f;
grids = {'LPG*', 'LTG*'};
for g = 1:numel(grids)
    cfg = [];
    cfg.channel = grids(g);
    cfg.keepchannel = 'yes';
    cfg.elec = elec_acpc_fr;
    cfg.method = 'headshape';
    cfg.headshape = hull_lh;
    cfg.warp = 'dykstra2012';
    cfg.feedback = 'yes';
elec_acpc_fr = ft_electroderealign(cfg);
end
```
24 Using the command below, visualize the cortex and electrodes together and examine whether they show the expected behavior (Fig. 3).

```matlab
ft_plot_mesh(pial_lh);
ft_plot_sens(elec_acpc_fr);
view([-55 10]);
material dull;
lighting gouraud;
camlight;
```

▲ CRITICAL STEP Accuracy of the re-alignment operation is important for obtaining precise knowledge of the location of the electrode and the part of the brain from which the local electrophysiological signal has originated. It is also important for correctly placing the electrodes in an anatomical context at Step 33.

? TROUBLESHOOTING

25 Using the command below, save the updated electrode information to a file.

```matlab
save([subjID '_elec_acpc_fr.mat'], 'elec_acpc_fr');
```

(Optional) Volume-based registration ● Timing ~2 min

▲ CRITICAL FieldTrip supports looking up the anatomical or functional labels corresponding to the electrodes in a number of atlases, including the AFNI Talairach–Tournoux atlas, the AAL atlas, the BrainWeb dataset, the JuBrain Cytoarchitectonic Atlas, the VTPM atlas, and the Brainnetome Atlas, in addition to the subject-tailored Desikan–Killiany and Destrieux atlases produced by FreeSurfer. Given that no two electrodes end up in exactly the same location across subjects because of inter-individual variability in electrode coverage and brain anatomy, atlases are particularly useful for the systematic combination of neural activity from different subjects in a so-called ROI analysis. With the exception of the FreeSurfer-based atlases (Desikan–Killiany and Destrieux), the atlases are in MNI coordinate space. If using an atlas in MNI coordinate space, spatially normalize the electrodes by following Steps 26 and 27.

26 To generalize the electrode coordinates to other brains or MNI-based neuroanatomical atlases at Step 33, register the subject’s brain to the standard MNI brain using the command below. The volume-based registration technique considers the overall geometry of the brain and can be used for the spatial normalization of all types of electrodes, whether at depth or on the surface.

```matlab
cfg = [];
cfg.nonlinear = 'yes';
cfg.spmversion = 'spm12';
fsmri_mni = ft_volumenormalise(cfg, fsmri_acpc);
```

27 Use the resulting deformation parameters to obtain the electrode positions in standard MNI space with the command below.

```matlab
elec_mni_frv = elec_acpc_fr;
elec_mni_frv.elecpos = ft_warp_apply(fsmri_mni.params, elec_acpc_fr.elecpos, 'individual2sn');
elec_mni_frv.chanpos = ft_warp_apply(fsmri_mni.params, elec_acpc_fr.chanpos, 'individual2sn');
elec_mni_frv.coordsys = 'mni';
```

---

**Fig. 3 | Brain-shift compensation.** In some patients, compensation for electrode displacement due to brain shift after implantation may be necessary. In this particular case, a subdural hygroma at the top of the brain caused severe electrode displacement in a direction opposite to the more commonly observed inward shift (left). Re-aligning electrode grids to the cortical surface can compensate for electrode displacement due to brain shift (right). The thin black lines indicate each electrode’s path from its localized origin on the left to its projected location on the right.
Using the command below, visualize the cortical mesh extracted from the standard MNI brain along with the spatially normalized electrodes and examine whether they show the expected behavior given the electrodes’ original anatomical locations (top right in Fig. 4).

**CRITICAL STEP** Accuracy of the volume-based registration is important for group-level analysis of brain activity and for correctly overlaying the electrode positions with a brain atlas at Step 33.

```matlab
load(<path to fieldtrip/template/anatomy/surface_pial_left.mat>);
ft_plot_mesh(mesh);
ft_plot_sens(elec_mni_frv);
view([-90 20]);
material dull;
lighting gouraud;
camlight;
```

**TROUBLESHOOTING**

Using the command below, save the normalized electrode information to a file.

```matlab
save([subjID '_elec_mni_frv.mat'], 'elec_mni_frv');
```

Surface-based registration (optional for surface electrodes) **Timing -2 min**

To enable the generalization of the electrode coordinates to other brains using the surface-based registration technique, map the electrodes onto FreeSurfer’s fsaverage brain with the command below. The surface-based registration technique considers only the curvature patterns of the cortex and thus can be used for the spatial normalization of electrodes located on or near the cortical surface. In the example case, this pertains to all electrodes of the left parietal and temporal grids.

```matlab
cfg = [];
cfg.channel = {'LPG*', 'LTG*'};
cfg.elec = elec_acpc_fr;
cfg.method = 'headshape';
cfg.headshape = <path to freesurfer/surf/lh.pial>;
cfg.warp = 'fsaverage';
cfg.fshome = <path to freesurfer home directory>;

elec_fsavg_frs = ft_electroderealign(cfg);
```

Using the command below, visualize FreeSurfer’s fsaverage brain, along with the spatially normalized electrodes, and examine whether they show the expected behavior (bottom right in Fig. 4).

**CRITICAL STEP** Accuracy of the surface-based registration is important for group-level analysis of cortical activity.

```matlab
fs_pial_lh = ft_read_headshape(<path to fshome/subjects/fsaverage/surf/lh.pial>);
fs_pial_lh.coordsys = 'fsaverage';
```
32 Using the command below, save the normalized electrode information to a file.

```matlab
save(['subjID '_elec_fsavg_frs.mat'], 'elec_fsavg_frs');
```

(Optional) Anatomical labeling ★ Timing ~1 min

33 Import the brain atlas of interest, e.g., the AAL atlas, into the MATLAB workspace using the below command.

```matlab
atlas = ft_read_atlas('path to fieldtrip/template/atlas/aal/ROI_MNI_V4.nii');
```

34 Using the command below, look up the corresponding anatomical label of an electrode of interest, e.g., electrode LHH1, targeting the left hemisphere’s hippocampus.

```matlab
cfg = [];
cfg.roi = elec_mni_frv.chanpos(match_str(elec_mni_frv.label, 'LHH1'),:);
cfg.atlas = atlas;
cfg.inputcoord = 'mni';
cfg.output = 'label';
lables = ft_volumelookup(cfg, atlas);
[-~, indx] = max(lables.count);
lables.name(indx) =
'ParaHippocampal_L'
```

Supplementary Data 3 is a tool that automatically overlays all channels in an electrode structure with all of the atlases listed above and stores the resulting anatomical labels in an Excel table (e.g., SubjectUCI29_electable.xlsx in the online repository).

Troubleshooting

Preprocessing of the neural recordings ★ Timing ~10 min

35 Using the command below, define the trials, that is, the segments of data that will be used for further processing and analysis. This step produces a matrix, `cfg.trl`, containing for each segment the begin and end sample in the recording file. In the case of the example provided in the shared data, the segments of interest begin 400 ms before tone onset, are marked with a ‘4’ in the trigger channel, and end 900 ms thereafter.

```matlab
cfg = [];
cfg.dataset = 'path to recording file';
cfg.trialdef.eventtype = 'TRIGGER';
cfg.trialdef.eventvalue = 4;
cfg.trialdef.prestim = 0.4;
cfg.trialdef.poststim = 0.9;
cfg = ft_definetrtrial(cfg);
```

36 Using the command below, import the data segments of interest into the MATLAB workspace and filter the data for high-frequency and power-line noise (type `help ft_preprocessing` for filtering options).

```matlab
cfg.demean = 'yes';
cfg.baselinewindow = 'all';
cfg.lpfilt = 'yes';
cfg.lpfreq = 200;
cfg.padding = 2;
cfg.padtype = 'data';
cfg.bsfilter = 'yes';
cfg.bsftord = 3;
cfg.bsfreq = [59 61; 119 121; 179 181];
data = ft_preprocessing(cfg);
```
37 Examine whether the variables in the output data structure match the recording and preprocessing parameters, i.e., the sampling rate \((f_{\text{sample}})\), number of recording channels \((\text{label})\), and segmentation into the experiment’s 26 trials \((\text{trial})\,\text{and their respective time axes in } \text{time})\) by typing \texttt{data} in the command window.

```matlab
    data =
    label: {152x1 cell}
    time: {1x26 cell}
    trial: {1x26 cell}
    fsample: 5000
    sampleinfo: [26x2 double]
    cfg: [1x1 struct]
```

38 Using the command below, add the \texttt{elec} structure originating from the anatomical workflow and save the preprocessed electrophysiological data to a file.

```matlab
    data.elec = elec_acpc_fr;
    save([subjID '_data.mat'], 'data');
```

The advantage of adding the electrode information at this stage is that it will be kept consistent with the neural data going forward, as when applying the same montage used for the neural recordings to the channel positions.

39 Inspect the neural recordings using \texttt{ft.databrowser} with the command below and identify channels or segments of noninterest, for instance, segments containing signal artifacts or (in this case) epileptiform activity. Mark the poor-quality segments by drawing a box around the corrupted signal. Write down the labels of poor-quality channels.

▲ CRITICAL STEP Identifying poor-quality channels is important for avoiding the contamination of other channels during remontaging in Step 41.

```matlab
    cfg = [];
    cfg.viewmode = 'vertical';
    cfg = ft.databrowser(cfg, data);
```

40 Remove any poor-quality segments marked in the above step using the command below.

```matlab
    data = ft.rejectartifact(cfg, data);
```

41 Using the code below, remontage the cortical grids to a common average reference in order to remove noise that is shared across all channels.

```matlab
    cfg = [];
    cfg.channel = {'LPG*', 'LTG*'};
    cfg.reref = 'yes';
    cfg.refchannel = 'all';
    cfg.refmethod = 'avg';
    reref_grids = ft.preprocessing(cfg, data);
```

Box 4 provides background information on remontaging. Poor-quality channels identified in Step 39 can be excluded from this step by adding those channels to \texttt{cfg.channel} with a minus prefix. That is, use \texttt{cfg.channel} = {'LPG*', 'LTG*', '-LPG1'} to exclude the LPG1 channel from the list of LPG and LTG channels.

---

**Box 4 | Remontaging**

The recorded electrophysiological signals are a mixture of the signal-of-interest and noise, both neural and non-neural. The main objective of the preprocessing of the neural recordings is to improve the signal-to-noise ratio of the data while optimally preparing it for follow-up analysis. Remontaging to a different referencing scheme, also known as a montage, may aid in the removal of noise that is shared across multiple channels. The common average re-referencing technique, for instance, involves taking the average potential from all channels and subtracting this global noise estimate from the potential in each channel\(^{49,71–74}\). We demonstrated how to apply this technique to the cortical grid electrodes in our example case.

Depth electrodes, located inside the brain and using differently sized and shaped contact points, have a different sensitivity distribution and capture different types of activity and levels of noise. Currently, no consensus exists on the preferred montage for depth-electrode recordings and, thus, which electrodes to use as references\(^{75–78}\). White-matter signals may not be as silent as one would intuitively expect, and bipolar signals, despite being relatively clean, miss out on activity that had the same amplitude on the two consecutive electrodes before their combination\(^{5,79}\). Different options may need to be tested and evaluated per case, taking into account the purpose of any follow-up analysis\(^{7}\). For instance, see ref.\(^{57}\) for a discussion of connectivity analysis in relation to the referencing scheme.
Apply a bipolar montage to the depth electrodes. This can be done by repeating Step 41 but selecting single-channel labels for `cfg.channel` and `cfg.refchannel`. Alternatively, it can be done using the code below, containing `cfg.refmethod = 'bipolar'`, which automatically takes bipolar derivations of consecutive channels.

```matlab
depths = {'RAM*', 'RHH*', 'RTH*', 'ROC*', 'LAM*', 'LHH*', 'LTH*'};
for d = 1:numel(depths)
    cfg = [];
    cfg.channel = ft_channelselection(depths{d}, data.label);
    cfg.reref = 'yes';
    cfg.refchannel = 'all';
    cfg.refmethod = 'bipolar';
    cfg.updatesens = 'yes';
    reref_depths{d} = ft_preprocessing(cfg, data);
end
```

New channel labels in the output indicate the bipolar origin of the data, e.g., `'RAM1–RAM2'`, `'RAM2–RAM3'`, and so on. By specifying `cfg.updatesens = 'yes'`, the same bipolar montage is automatically applied to the channel positions, with the resulting `chanpos` field containing the mean locations of all electrode pairs that constitute a bipolar channel. More elaborate schemes can be created by the specification of weight matrices using `cfg.montage` that define how existing channels should be combined into new channels (type `help ft_apply_montage` for further guidance).

Combine the data from both electrode types into one data structure for the ease of further processing using the code below.

```matlab
cfg = [];
cfg.appendsens = 'yes';
reref = ft_appenddata(cfg, reref_grids, reref_depths{:});
```

Using the command below, save the re-referenced data to a file.

```matlab
save([subjID '_reref.mat'], reref);
```

(Optional) Time–frequency analysis

Decompose the signal in time and frequency using time-resolved Fourier-based spectral decomposition. The below command samples the neural activity using `cfg.toi` throughout the 300-ms baseline period before tone onset at 0 ms and until after the approximate button-press time at 700 ms. At each time point, the spectral content is estimated using 200-ms windows (`cfg.t_ftimwin`) for a broad range of frequencies specified with `cfg.foi`.

```matlab
cfg = [];
cfg.method = 'mtmconvol';
cfg.toi = -.3:0.01:.8;
cfg.t_ftimwin = ones(length(cfg.foi),1).*0.2;
cfg.foi = 5:5:200;
cfg.taper = 'hanning';
cfg.output = 'pow';
cfg.keeptrials = 'no';
freq = ft_freqanalysis(cfg, reref);
```

The settings for spectral decomposition depend on the clinical or research question at hand and contingencies in the experimental paradigm. Given that task-related changes in high-frequency-band activity (70–150 Hz) are the focus of this analysis, relatively short time windows of 200 ms are used. This time window imposes a frequency resolution of 5 Hz, given that it fits only integer multiples of that frequency's cycle. The 200-ms time window captures 20 cycles of a 100-Hz rhythm, which is well over the three cycles minimally required to unambiguously recover the spectral information. To reliably capture lower frequencies, the length of the time window may need to be increased, data-segment length permitting. Finally, note that `cfg.keeptrials` is set to 'no' (as it is by default), which results in the average spectral content across all trials being returned (type `help ft_freqanalysis` for further guidance).

Using the command below, save the time–frequency data to a file.

```matlab
save([subjID '_freq.mat'], 'freq');
Interactive plotting

47 For an anatomically informed exploration of the multidimensional outcome of an analysis, create a layout based on the 3D electrode locations, using the command below.

```matlab
cfg = []; 
cfg.headshape = 'pial_lh';
cfg.projection = 'orthographic';
cfg.channel = {'LPG*', 'LTG*'};
cfg.viewpoint = 'left';
cfg.mask = 'convex';
cfg.boxchannel = {'LTG30', 'LTG31'};
lay = ft_prepare_layout(cfg, freq);
```

This layout is a symbolic representation in which the channels are projected on the 2D medium offered by paper or a computer screen. The layout is complemented by an automatic outline of the cortical sheet that is specified in `cfg.headshape` the command. The `cfg.boxchannel` option allows selection of channels whose 2D distances are used to determine the plotting box sizes in the following step.

48 Using the command below, express the time–frequency representation of neural activity at each channel in terms of the relative change in activity from the baseline interval. The baseline interval from −100 to 0 ms is excluded, given that power spectral data points in this interval were estimated using 200-ms time windows that extend into the task period.

```matlab
cfg = []; 
cfg.baseline = [-.3 -.1];
cfg.baselinetype = 'relchange';
freq_blc = ft_freqbaseline(cfg, freq);
```

49 Using the command below, visualize the time–frequency representations overlaid on the 2D layout.

```matlab
cfg = [];
cfg.layout = lay;
cfg.showoutline = 'yes';
ft_multiplotTFR(cfg, freq_blc);
```

The generated figure is interactive, so that selecting a group of channels will launch another figure representing the average time–frequency representation over those channels (Fig. 5). Selecting a certain frequency and time range in that time–frequency representation will launch yet another

---

**Fig. 5 | Interactive plotting.** Fast browsing through various anatomically informed representations of the neural data can help address the multidimensionality of intracranial EEG data. Selecting a group of channels in the main figure on the left will launch another figure representing the average time–frequency representation over those channels (middle). Selecting a certain frequency and time range in that time–frequency representation will launch yet another figure showing the topographical distribution of activity in the selected interval (right), and so on.
Figure showing the topographical distribution of activity in the selected interval, and so on (see also Supplementary Video 5 for guidance).

**ECoG data representation** ● **Timing -1 min**

50 For an anatomically realistic representation of cortical activity, overlay a surface model of the neocortex with the spatial distribution of high-frequency-band activity. First, extract mean high-frequency-band activity during a time interval of interest with the command below.

```matlab
cfg = [];  
cfg.frequency = [70 150];  
cfg.avgoverfreq = 'yes';  
cfg.latency = [0 0.8];  
cfg.avgovertime = 'yes';  
freq_sel = ft_selectdata(cfg, freq_blc);
```

Using the command below, visualize the spatial distribution of high-frequency-band activity on a cortical mesh of the subject’s brain (Fig. 6).

```matlab
cfg = [];  
cfg.funparameter = 'powspctrm';  
cfg.funcolorlim = [-.5 .5];  
cfg.method = 'surface';  
cfg.interpmethod = 'sphere_weighteddistance';  
cfg.sphereradius = 8;  
cfg.camlight = 'no';  
ft_sourceplot(cfg, freq_sel, pial_lh);  
view([-90 20]);  
material dull;  
lighting gouraud;  
camlight;
```

By repeating Step 50 and this step with the time interval of interest specified with `cfg.latency` broken down in consecutive steps, the spatiotemporal dynamics of neural activity occurring in relation to known experimental structure and behavior can be observed (see Supplementary Video 6 for an example of results obtained from using the example dataset). Type `help getframe` for capturing and assembling time-lapse movies.

52 Using the command below, add the electrodes to the figure.

```matlab
ft_plot_sens(elec_acpc_fr);
```
53 For depth recordings, create an integrated representation of neural activity and anatomy by interpolating neural data from each bipolar channel in a spherical cloud, which can then be overlaid on a surface mesh of any deep-brain structure. First, create a volumetric mask of the regions of interest. The command below generates a mask for the right hippocampus and amygdala from the cortical parcellation and subcortical segmentation produced by FreeSurfer.

```matlab
atlas = ft_read_atlas('freesurfer/mri/aparc+aseg.mgz');
atlas.coordsys = 'acpc';
cfg = [];
cfg.inputcoord = 'acpc';
cfg.atlas = atlas;
cfg.roi = {'Right-Hippocampus', 'Right-Amygdala'};
mask_rha = ft_volumelookup(cfg, atlas);
```

54 Using the command below, create a triangulated and smoothed surface mesh on the basis of the volumetric masks.

```matlab
seg = keepfields(atlas, {'dim', 'unit', 'coordsys', 'transform'});
seg.brain = mask_rha;
cfg = [];
cfg.method = 'iso2mesh';
cfg.radbound = 2;
cfg.maxsurf = 0;
cfg.tissue = 'brain';
cfg.numvertices = 1000;
cfg.smooth = 3;
mesh_rha = ft_prepare_mesh(cfg, seg);
```

55 Using the command below, identify the subcortical electrodes of interest.

```matlab
cfg = [];
cfg.channel = {'RAM*', 'RTH*', 'RHH*'};
freq_sel2 = ft_selectdata(cfg, freq_sel);
```

---

**Fig. 7 | SEEG data representation.** Task-induced high-frequency-band activity (70–150 Hz) relative to a baseline interval, plotted as point clouds around a triangulated mesh of a different subject’s right amygdala and hippocampus combined. The neural activity is averaged across the frequency band of interest and the time interval between 0 and 800 ms following the onset of a tone that required the subject to prepare and execute a button press with the right hand. The size and color of the point clouds are scaled according to the percentage of high-gamma-band activity modulation from baseline power. The 2D planes on the right correspond to the slices indicated by the black lines in the image on the left.
56 Using the commands below, interpolate the high-frequency-band activity in the bipolar channels on a spherical cloud around the channel positions while overlaying the neural activity with the above mesh. (Supplementary Figure 1 shows examples of the cloud types currently available to plot.)

```matlab
cfg = [];  
cfg.funparameter = 'powspctrm';  
cfg.funcolorlim = [-.5 .5];  
cfg.method = 'cloud';  
cfg.slice = '3d';  
cfg.n_slices = 2;  
cfg.facealpha = .25;  
ft_sourceplot(cfg, freq_sel2, mesh_rha);  
view([120 40]);  
lighting gouraud;  
camlight;
```

By repeating the above command for neural data corresponding to consecutive time intervals, similar to the process outlined in Step 51, it becomes feasible to create time-lapse movies of the spatiotemporal dynamics of deep-brain activity. (Supplementary Video 7 shows the spatiotemporal evolution of epileptiform activity obtained from a dataset from a different subject.)

57 To create a more definitive image of the neural activity at particular positions, use the command below to generate 2D slices through the 3D representations. This combination provides the most complete and integrated representation of neural and anatomical data (Fig. 7).

```matlab
cfg.slice = '2d';
ft_sourceplot(cfg, freq_sel2, mesh_rha);
```

## Troubleshooting

Troubleshooting advice can be found in Table 1.

### Table 1 | Troubleshooting table

| Step | Problem | Possible reason | Solution |
|------|---------|----------------|----------|
| 7 | Unsatisfactory quality of cortical surfaces | Insufficient quality of the MRI | Repeat Step 6 on another MRI or manually correct the topological defects (see FreeSurfer website) |
| 14 | Severe misalignment of CT and MRI | Failure of the automatic CT conversion in Step 12 | Use educated guesses of the commissure locations to directly align to the ACPC system in Step 11 |
| 14 | Imperfect alignment of CT and MRI | A left–right flip of either the MRI or the CT | Re-examine the native orientations of the MRI and CT in Steps 3 and 10, and redo the preprocessing of the affected scan |
| 14 | Imperfect alignment of CT and MRI | MRI and CT contain different head anatomies | Repeat Step 13 with a different cost function (type help ft_volumerealign) |
| 17 | Electrodes are hard to identify in the 2D orthogonal plot | Cortical grid orientation is not aligned with any of the 2D planes | Identify electrodes in the 3D scatter figure (check the scatter checkbox) |
| 19 | Electrodes are missing or mislabeled | Human error during electrode placement | Revisit Step 17 to correct while specifying `cfg.elec = elec_acpc_f` to include previously localized electrodes |
| 24 | Severe deformation of the electrode grid | Incorrect pairing of neighboring electrodes in space | Repeat Step 23 with alternative settings (type help ft_electroderealign) |
| 28 | Severe displacement of electrodes | Insufficient quality of the MRI | Repeat Step 26 with an alternative cost function or template version (type help ft_volumenormalise) |
| 34 | No anatomical label found | No overlap of electrode position with any anatomical mask | Increase the search radius around the electrode by increasing the `cfg.maxqueryrange` value (type help ft_volumelookup) |

## Timing

- Step 1, specification of subject ID: ~1 min
- Steps 2–5, preprocessing of the anatomical MRI: ~2 min
- Steps 6–8, (optional) cortical surface extraction with FreeSurfer: ~10 h
- Steps 9–12, preprocessing of the anatomical CT: ~2 min
- Steps 13–15, fusion of the CT with the MRI: ~3 min
Steps 16–20, electrode placement: ~15 min
Steps 21–25, (optional) brain-shift compensation: ~5 min
Steps 26–29, (optional) volume-based registration: ~2 min
Steps 30–32, (optional) surface-based registration: ~2 min
Steps 33 and 34, (optional) anatomical labeling: ~1 min
Steps 35–44, preprocessing of the neural recordings: ~10 min
Steps 45 and 46, (optional) time–frequency analysis: ~2 min
Steps 47–49, interactive plotting: ~3 min
Steps 50–52, ECoG data representation: ~1 min
Steps 53–57, SEEG data representation: ~2 min
Box 1, getting started with FieldTrip: ~1 min
Box 2, example data set
Box 3, coordinate system determination: ~1 min
Box 4, remontaging

Anticipated results

Upon completion of the protocol, an integrated representation of neural and anatomical data should be obtained. The exact results ultimately depend on the clinical or research question at hand, contingencies in the experimental paradigm, and decisions made during the execution of the protocol. For example, the worked example we provide here demonstrates the spatiotemporal neural dynamics that occur in relation to known experimental structure and relatively simple behavior, specifically the pressing of a button with the right hand when hearing a target tone (results obtained from the example dataset are shown in Figs. 5–7 and Supplementary Video 6). However, with small adaptations of the protocol, it is feasible to track the spatiotemporal evolution of epileptiform activity with high precision (e.g., Supplementary Video 7) or to perform group-level investigations of fine-grained emotion- or language-related neural dynamics in the human hippocampus20,24. A precise fusion of the anatomical images with the electrophysiological data is key to reproducible analyses and findings. Hence, it is important to examine the outcome of each part of the analysis as described in the Procedure and illustrated by Figs. 3 and 4.

Further information on experimental design is available in the Life Sciences Reporting Summary.

References

1. Buzsaki, G., Anastassiou, C. A. & Koch, C. The origin of extracellular fields and currents--EEG, ECoG, LFP and spikes. Nat. Rev. Neurosci. 13, 407–420 (2012).
2. Malmivuo, J. & Plonsey, R. Bioelectromagnetism: Principles and Applications of Bioelectric and Biomagnetic Fields. Bioelectromagnetism: Principles and Applications of Bioelectric and Biomagnetic Fields (Oxford University Press, New York, 2012).
3. Brunner, P. et al. A practical procedure for real-time functional mapping of eloquent cortex using electrocorticographic signals in humans. Epilepsy Behav. 15, 278–286 (2009).
4. Ritaccio, A. et al. Proceedings of the fifth international workshop on advances in electrocorticography. Epilepsy Behav. 41, 183–92 (2014).
5. Lachaux, J.-P., Axmacher, N., Mormann, F., Halgren, E. & Crone, N. E. High-frequency neural activity and human cognition: past, present and possible future of intracranial EEG research. Prog. Neurobiol. 98, 279–301 (2012).
6. Friston, J. A. & Friston, K. Multimodal image coregistration and partitioning - a unified fFramework. Neuroimage 6, 209–217 (1997).
7. Jenkinson, M., Bannister, P., Brady, M. & Smith, S. Improved optimization for the robust and accurate linear registration and motion correction of brain images. Neuroimage 17, 825–841 (2002).
8. Cox, R. W. AFNI: software for analysis and visualization of functional magnetic resonance neuroimages. Comput. Biomed. Res. 29, 162–173 (1996).
9. Papademetris, X. et al. BioImage suite: an integrated medical image analysis suite: an update. Insight J. 2006, 209 (2006).
10. Azarion, A. A. et al. An open-source automated platform for three-dimensional visualization of subdural electrodes using CT-MRI coregistration. Epilepsia 55, 2028–2037 (2014).
11. Blenkmann, A. O. et al. iElectrodes: a comprehensive open-source Toolbox for depth and subdural grid electrode localization. Front. Neuroinform. 11, 14 (2017).
12. Groppe, D. M. et al. iELVis: an open source MATLAB toolbox for localizing and visualizing human intracranial electrode data. J. Neurosci. Methods 281, 40–48 (2017).
13. Kubanek, J. & Schalk, G. NeuralAct: a tool to visualize electrocortical (ECoG) activity on a three-dimensional model of the cortex. Neuroinformatics 13, 167–174 (2015).
14. Qin, C. et al. Automatic and precise localization and cortical labeling of subdural and depth intracranial electrodes. Front. Neuroinform. 11, 1–10 (2017).
15. Hill, N. J. et al. Recording human electrocorticographic (ECoG) signals for neuroscientific research and real-time functional cortical mapping. J. Vis. Exp. https://doi.org/10.3791/3993 (2012).
16. LaPlante, R. A. et al. The interactive electrode localization utility: software for automatic sorting and labeling of intracranial subdural electrodes. Int. J. Comput. Assist. Radial. Surg. 12, 1829–1837 (2017).
17. Branco, M. P. et al. ALICE: a tool for automatic localization of intra-cranial electrodes for clinical and high-density grids. J. Neurosci. Methods 301, 43–51 (2018).
18. Eglen, S. J. et al. Toward standard practices for sharing computer code and programs in neuroscience. Nat. Neurosci. 20, 770–773 (2017).
19. Delorme, A. & Makeig, S. EEGLAB: an open source toolbox for analysis of single-trial EEG dynamics including independent component analysis. J. Neurosci. Methods 134, 9–21 (2004).
20. Zheng, J. et al. Amygdala-hippocampal dynamics during salient information processing. Proc. Natl. Acad. Sci. USA 113, 11366–11371 (2016).
21. Hermes, D., Miller, K. J., Noordmans, H. J., Vansteensel, M. J. & Ramsey, N. F. Automated electrocorticographic electrode localization on individually rendered brain surfaces. J. Neurosci. Methods 185, 293–298 (2010).
22. Dalal, S. S. et al. Localization of neurosurgically implanted electrodes via photograph-MRI-radiograph coregistration. J. Neurosci. Methods 174, 106–115 (2008).
23. Yang, A. I. et al. Localization of dense intracranial electrode arrays using magnetic resonance imaging. Neuroimage 63, 157–165 (2012).
24. Onofrey, J. A., Staib, L. H. & Papademetris, X. Learning intervention-induced deformations for non-rigid MR-CT registration and electrode localization in epilepsy patients. Neuroimage Clin. 10, 291–301 (2016).
25. Pieters, T. A., Conner, C. R. & Tandon, N. Recursive grid partitioning on a cortical surface model: an optimized technique for the localization of implanted subdural electrodes. J. Neurosurg. 118, 1086–1097 (2013).
26. Stieglitz, L. H. et al. Improved localization of implanted subdural electrode contacts on magnetic resonance imaging with an elastic image fusion algorithm in an invasive electroencephalography recording. Clin. Neurosurg. 10, 506–513 (2014).
27. Brang, D., Dai, Z., Zheng, W. & Towle, V. L. Registering imaged ECoG electrodes to human cortex: a geometry-based technique. J. Neurosci. Methods 273, 64–73 (2016).
28. Dykstra, A. R. et al. Individualized localization and cortical surface-based registration of intracranial electrodes. Neuroimage 59, 3563–3570 (2012).
29. Khodagholy, D. et al. Organic electronics for high-resolution electrocorticography of the human brain. Sci. Adv. 2, 1–9 (2016).
30. Brang, D., Towle, V. L., Hartov, A., Kennedy, F. E., Miga, M. I. & Paulsen, K. D. Intraoperative brain shift and deformation: a quantitative analysis of cortical displacement in 28 cases. Neurosurgery 43, 749–758 (1998).
31. Miyagi, Y., Shima, F. & Sasaki, T. Brain shift: an error factor during implantation of deep brain stimulation electrodes. J. Neurosurg. 107, 989–97 (2007).
32. Hastreiter, P. et al. Strategies for brain shift evaluation. Med. Image Anal. 8, 447–464 (2004).
44. LaViolette, P. S. et al. Three-dimensional visualization of subdural electrodes for presurgical planning. *Oper. Neurosurg.* 68 https://doi.org/10.1227/NEU.0b013e31820783ba (2011).
45. Sweet, J. A., Hdeib, A. M., Sloan, A. & Miller, J. P. Depths and grids in brain tumors: Implantation strategies, techniques, and complications. *Epilepsia* 54, 66–71 (2013).
46. Kovalév, D. et al. Rapid and fully automated visualization of subdural electrodes in the presurgical evaluation of epilepsy patients. *Am. J. Neuroradiol.* 26, 1078–1083 (2005).
47. Wang, P. T. et al. A co-registration approach for electrocorticogram electrode localization using post-implantation MRI and CT of the head. in *Proc. International. IEEE/EMBS Conference on Neural Engineering, NER* 525–528 https://doi.org/10.1109/NER.2013.6695987 (2013).
48. Schulze-Bonhage, A. H. J. et al. Visualization of subdural strip and grid electrodes using curvilinear reformatting of 3D MR imaging data sets. *Am. J. Neuroradiol.* 23, 400–403 (2002).
49. Boatman-Reich, D. et al. Quantifying auditory event-related responses in multichannel human intracranial recordings. *Front. Comput. Neurosci.* 4, 4 (2010).
50. Staresina, B. P. et al. Hierarchical nesting of slow oscillations, spindles and ripples in the human hippocampus during sleep. *Nat. Neurosci.* 18, 1679–1686 (2015).
51. Manning, J. R., Jacobs, J., Fried, I. & Kahana, M. J. Broadband shifts in local anatomical parcellation of the MNI MRI single-subject brain. *Cereb. Cortex* 25, 1325–1335 (2005).
52. Dubois, T., Akbar, A. & Colliou, J.-M. Automated multi-modality image registration based on information theory. *Brain Warping* 170 (2008).
53. Tzourio-Mazoyer, N. et al. Automated anatomical labeling of activations in SPM using a macroscopic anatomical parcellation of the MNI MRI single-subject brain. *Neuroimage* 15, 273–289 (2002).
54. Cocosco, C. A., Kollokian, V., Kwan, R. K., Pike, G. B. & Evans, A. C. BrainWeb : online interface to a 3D MRI simulated brain database. *Proc. 3rd Int. Conf. Funct. Mapp. Hum. Brain* 3, S425 (1997). in.
55. Eickhoff, S. B. et al. A new SPM toolbox for combining probabilistic cytoarchitectonic maps and functional imaging data. *Neuroimage* 25, 1325–1335 (2005).
56. Wang, L., Murezcz, R. E. B., Arcaro, M. J. & Kastner, S. Probabilistic maps of visual topography in human cortex. *Cereb. Cortex* 25, 3911–3931 (2015).
57. Fan, L. et al. The human brainnetome atlas: a new brain atlas based on connectional architecture. *Cereb. Cortex* 26, 3508–3526 (2016).
58. Desikan, R. S. et al. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage* 31, 968–980 (2006).
59. Destrieux, C., Fischl, B., Dale, A. & Halgren, E. Automatic parcellation of human cortical gyri and sulci using standard anatomical nomenclature. *Neuroimage* 53, 1–15 (2010).
60. Ashburner, J. & Friston, K. J. Nonlinear spatial normalization using basis functions. *Hum. Brain Mapp.* 7, 254–266 (1999).
61. Bigdely-Shamlo, N., Mullen, T., Kothe, C., Su, K.-M. & Robbins, K. A. The PREP pipeline: standardized preprocessing for large-scale EEG analysis. *Front. Neuroinform.* 9, 16 (2015).
62. Liu, Y., Coon, W. G., Pesters, A., de Brunner, P. & Schalk, G. The effects of spatial filtering and artifacts on electrocorticographic signals. *J. Neural Eng.* 12, S6008 (2015).
63. Dienes, J. Issues in the application of the average reference: review, critiques, and recommendations. *Behav. Res. Methods, Instrum., Comput.* 30, 34–43 (1998).
64. Ludwig, K. A. et al. Using a common average reference to improve cortical neuron recordings from microelectrode arrays. *J. Neurophysiol.* 101, 1679–1689 (2009).
75. Trongnetrpunya, A. et al. Assessing Granger causality in electrophysiological data: removing the adverse effects of common signals via bipolar derivations. *Front. Syst. Neurosci.* **9**, 189 (2015).

76. Shirhatti, V., Borthakur, A. & Ray, S. Effect of reference scheme on power and phase of the local field potential. *Neural Comput.* **882–913** https://doi.org/10.1162/NECO (2016).

77. Arnulfo, G., Hirvonen, J., Nobili, L., Palva, S. & Palva, J. M. Phase and amplitude correlations in resting-state activity in human stereotactical EEG recordings. *Neuroimage* **112**, 114–127 (2015).

78. Zaveri, H. P., Duckrow, R. B. & Spencer, S. S. On the use of bipolar montages for time-series analysis of intracranial electroencephalograms. *Clin. Neurophysiol.* **117**, 2102–2108 (2006).

79. Mercier, M. R. et al. Evaluation of cortical local field potential diffusion in stereotactic electroencephalography recordings: a glimpse on white matter signal. *Neuroimage* **147**, 219–232 (2017).

Acknowledgements
The authors thank the patient for participation and C.R. Holdgraf, V. Rangarajan, C.W. Hoy, J. Kam, L. Bellier, R. Helfrich, R. Jimenez, E. Gerber, A. Blenkmann, J. Lubell, and M. Pereira for fruitful discussions. The authors are also grateful to the present and former FieldTrip core developers, as well as the greater FieldTrip community, for contributing code, documentation, and expertise that have made this protocol possible. A.S. was supported by Rubicon grant 446-14-007 from NWO and Marie Skłodowska-Curie Global Fellowship 658868 from the European Union; R.v.d.M. by R01 MH095984-05S1 from the NIMH; J.-M.S. by VIDI 864-14-011 from NWO, R.T.K. by R37 NS21135 from NINH, and R.O. by Marie Skłodowska-Curie Innovative Training Networks 641652 from the European Union.

Author contributions
A.S., S.G., R.v.d.M., J.-M.S., and R.O. developed the protocol. G.P. contributed the algorithm for brain-shift compensation. J.J.L. provided access and guidance in the data acquisition. A.S., S.G., J.-M.S., R.T.K., and R.O. wrote the paper, and R.v.d.M., C.D., I.S., G.P., and J.J.L., provided substantial editorial revisions.

Competing interests
The authors declare no competing interests.

Additional information
Supplementary information is available for this paper at https://doi.org/10.1038/s41596-018-0009-6.

Reprints and permission information is available online at www.nature.com/reprints

Correspondence and requests for materials should be addressed to A.S.

Publisher’s note: Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Published online: 9 July 2018

Related links
1. Zheng, J. et al. *Nat. Commun.* **8**, 14413 (2017): http://dx.doi.org/10.1038/ncomms14413
2. Piai et al. *Proc. Natl. Acad. Sci. USA* **113**, 11366–11371 (2016): http://dx.doi.org/10.1073/pnas.1603312113
Reporting Summary

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

Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

- The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- The statistical test(s) used AND whether they are one- or two-sided
- Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- A description of all covariates tested
- A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- For null hypothesis testing, the test statistic (e.g. F, t, r) with confidence intervals, effect sizes, degrees of freedom and P value noted
  Give P values as exact values whenever suitable.
- For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- Estimates of effect sizes (e.g. Cohen’s d, Pearson’s r), indicating how they were calculated
- Clearly defined error bars
  State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on statistics for biologists may be useful.

Software and code

Policy information about availability of computer code

Data collection
All neural recordings were acquired using a Nihon Kohden recording system with a JE-120A amplifier (Nihon Kohden Corporation, Tokyo, Japan), analog-filtered above 0.01 Hz, and digitally sampled at 5 KHz. The sequence imaging parameters used for the MRI were MP-RAGE, 256x256x176mm with 1x1x1mm isotropic voxels.

Data analysis
All data were analyzed using the FieldTrip toolbox as detailed in the protocol.

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

Data
Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:
- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The original data set (after defacing the imaging data with ft_defacevolume) and the processed results are available for download from http://doi.org/11633/di.dccn.DSC_3015000.00_734. Raw DICOM images and recording files are not shared to protect the subject’s identity.

Field-specific reporting

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

- [ ] Life sciences
- [ ] Behavioural & social sciences

For a reference copy of the document with all sections, see nature.com/authors/policies/ReportingSummary-flat.pdf

Life sciences

Study design

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

- **Sample size**: 1 example dataset for demonstration of the protocol
- **Data exclusions**: None
- **Replication**: NA
- **Randomization**: NA
- **Blinding**: NA

Materials & experimental systems

Policy information about availability of materials

- [ ] Involved in the study
- [ ] Unique materials
- [ ] Antibodies
- [ ] Eukaryotic cell lines
- [ ] Research animals
- [ ] Human research participants

Human research participants

Policy information about studies involving human research participants

- **Population characteristics**: The (deidentified) example dataset is of an epilepsy patient implanted with electrodes for neurosurgical purposes

Method-specific reporting

- [ ] Involved in the study
- [ ] ChIP-seq
- [ ] Flow cytometry
- [ ] Magnetic resonance imaging

Magnetic resonance imaging

- **Experimental design**: NA
| Design specifications | NA |
|-----------------------|----|
| Behavioral performance measures | NA |

**Acquisition**

| Imaging type(s) | Structural |
|-----------------|------------|
| Field strength | 1.5T |
| Sequence & imaging parameters | MP-RAGE, 256x256x176mm with 1x1x1mm isotropic voxels |
| Area of acquisition | Head |
| Diffusion MRI | Not used |

**Preprocessing**

| Preprocessing software | FieldTrip and FreeSurfer |
|------------------------|--------------------------|
| Normalization | NA |
| Normalization template | NA |
| Noise and artifact removal | NA |
| Volume censoring | NA |

**Statistical modeling & inference**

| Model type and settings | NA |
|--------------------------|----|
| Effect(s) tested | NA |
| Specify type of analysis: | □ Whole brain □ ROI-based □ Both |
| Statistic type for inference | NA |
| Correction | NA |

**Models & analysis**

| n/a Involved in the study | □ Functional and/or effective connectivity |
|---------------------------|-------------------------------------------|
|                          | □ Graph analysis                           |
|                          | □ Multivariate modeling or predictive analysis |
### Experimental design

| Number | Description |
|--------|-------------|
| 1.     | Describe the experimental design. | The MRI is an anonymized structural scan of a patient |
| 2.     | Specify the number of blocks, trials or experimental units per session and/or subject, and specify the length of each trial or block (if trials are blocked) and interval between trials. | does not apply |
| 3.     | Describe how behavioral performance was measured. | No functional data was recorded with MRI |

### Acquisition

| Type | Description |
|------|-------------|
| 1.   | Imaging a. Specify the type(s) of imaging. | Structural scan |
|      | b. Specify the field strength (in Tesla). | 3T |
|      | c. Provide the essential sequence imaging parameters. | MP-RAGE, 256x256x176mm with 1x1x1mm isotropic voxels |
|      | d. For diffusion MRI, provide full details of imaging parameters. | does not apply |
| 2.   | State area of acquisition. | Head |

### Preprocessing

| Step | Description |
|------|-------------|
| 1.   | Describe the software used for preprocessing. | FieldTrip, SPM, FreeSurfer |
| 2.   | Normalization a. If data were normalized/standardized, describe the approach(es). | does not apply |
|      | b. Describe the template used for normalization/transformation. | does not apply |
| 3.   | Describe your procedure for artifact and structured noise removal. | does not apply |
| 4.   | Define your software and/or method and criteria for volume censoring, and state the extent of such censoring. | does not apply |

### Statistical modeling & inference

| Step | Description |
|------|-------------|
| 1.   | Define your model type and settings. | does not apply |
| 2.   | Specify the precise effect tested. | does not apply |
12. Analysis
   a. Specify whether analysis is whole brain or ROI-based. does not apply
   b. If ROI-based, describe how anatomical locations were determined.
   does not apply

13. State the statistic type for inference.
   (See Eklund et al. 2016.)
   does not apply

14. Describe the type of correction and how it is obtained for multiple comparisons.
   does not apply

15. Connectivity
   a. For functional and/or effective connectivity, report the measures of dependence used and the model details.
   does not apply
   b. For graph analysis, report the dependent variable and functional connectivity measure.
   does not apply

16. For multivariate modeling and predictive analysis, specify independent variables, features extraction and dimension reduction, model, training and evaluation metrics.
   does not apply