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TITLE:
Analyzing Neural Activity and Connectivity Using Intracranial EEG Data with SPM Software

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KEYWORDS:
cross-frequency coupling; dynamic causal modeling (DCM); face; gamma oscillation; inferior occipital gyrus; intracranial electroencephalography (EEG); time–frequency analysis.

SUMMARY:
We present two analytical protocols that can be used to analyze intracranial electroencephalography data using the Statistical Parametric Mapping (SPM) software: time–frequency statistical parametric mapping analysis for neural activity, and dynamic causal modeling of induced responses for intra- and inter-regional connectivity.

ABSTRACT
Measuring neural activity and connectivity associated with cognitive functions at high spatial and temporal resolutions is an important goal in cognitive neuroscience. Intracranial electroencephalography (EEG) can directly record electrical neural activity and has the unique potential to accomplish this goal. Traditionally, averaging analysis has been applied to analyze intracranial EEG data; however, several new techniques are available for depicting neural activity and intra- and inter-regional connectivity. Here, we introduce two analytical protocols we recently applied to analyze intracranial EEG data using the Statistical Parametric Mapping (SPM) software: time–frequency SPM analysis for neural activity and dynamic causal modeling of induced responses for intra- and inter-regional connectivity. We report our analysis of

intracranial EEG data during the observation of faces as representative results. The results revealed that the inferior occipital gyrus (IOG) showed gamma-band activity at very early stages (110 ms) in response to faces, and both the IOG and amygdala showed rapid intra- and inter-regional connectivity using various types of oscillations. These analytical protocols have the potential to identify the neural mechanisms underlying cognitive functions with high spatial and temporal profiles.

INTRODUCTION

Measuring neural activity and connectivity associated with cognitive functions at high spatial and temporal resolutions is one of the primary goals of cognitive neuroscience. However, accomplishing this goal is not easy. One popular method used to record neural activity is functional magnetic resonance imaging (MRI). Although functional MRI offers several advantages, such as a high spatial resolution at the millimeter level and non-invasive recording, a clear disadvantage of functional MRI is its low temporal resolution. In addition, functional MRI measures blood-oxygen-level-dependent signals, which only indirectly reflect electric neural activity. Popular electrophysiological methods, including electroencephalography (EEG) and magnetoencephalography (MEG), have high temporal resolutions at the millisecond level. However, they have relatively low spatial resolutions, because they record electric/magnetic signals at the scalp and must solve difficult inverse problems to depict brain activity.

Intracranial EEG can directly record electrical neural activity at high temporal (millisecond) and spatial (centimeter) resolutions[1]. This measure can provide valuable opportunities to understand neural activity and connectivity, although it has clear limitations (*e.g.*, measurable regions are restricted to clinical criteria). Several intracranial EEG studies have applied traditional averaging analysis to depict neural activity. Although averaging analysis can sensitively detect time-locked and low-frequency band activation, it cannot detect non-phase-locked and/or high-frequency (*e.g.,* gamma band) activation. In addition, functional neural coupling has not been analyzed in depth in the literature on intracranial EEG recordings. Several new techniques have been recently developed to depict neural activity and intra- and inter-regional connectivity in functional MRI and EEG/MEG recordings, which can be applied to analyze intracranial EEG data.

Here, we introduce analytical protocols that we have recently applied to analyze intracranial EEG data using the Statistical Parametric Mapping (SPM) software. First, to reveal when, and at which frequency, the brain regions could be activated, we performed time–frequency SPM analysis[2]. This analysis decomposes the time and frequency domains simultaneously using a continuous wavelet transform and appropriately corrects the family-wise error (FWE) rate in the time–frequency maps using the random field theory. Second, to reveal how brain regions communicate, we applied dynamic causal modeling (DCM) of induced responses[4]. DCM enables the investigation of effective connectivity (*i.e.,* the causal and directional influences among brain regions[5]). Although DCM was originally proposed as a tool for analyzing functional MRI data[5], DCM of induced responses has been extended to analyze the time-varying power spectra of electrophysiological signals[4]. This analysis allows the depiction of both intra- and inter-regional neural connectivity. Several neurophysiological studies have suggested that local intra-

regional computations and long-range inter-regional communication mainly use gamma- and theta-band oscillations, respectively, and their interactions (*e.g.*, entrainments) can be reflected by theta–gamma cross-frequency coupling[3,6–8]. This report focuses on the data analytical protocol; for an overview of background information[9,10] and recording protocols[11] of intracranial EEG, please refer to the literature.

PROTOCOL:

Our study was approved by the local institutional ethics committee.

1. Basic Information

Note: The analytical protocols can be applied to various types of data without any restrictions as to specific participants, electrodes, reference methods, or electrode locations. In our example, we tested six patients suffering from pharmacologically intractable focal epilepsy. We tested patients who had no epileptic foci in the regions of interest.

1.1. Record intracranial EEG data during the cognitive experiment at the target electrodes.

1.1.1. Implant depth electrodes using the stereotactic method[12].

1.1.2. Use subdural platinum electrodes (diameter: 2.3 mm) and depth platinum electrodes (diameter: 0.8 mm) to simultaneously measure cortical and subcortical activity, respectively.

1.1.3. Place reference electrodes on the surface of the skull of the midline dorsal frontal region, with the contacts of the electrodes facing away from the skull to avoid referential activation[12].

1.1.4. Amplify data, filter online (band pass: 0.5–300 Hz), and sample at 1000 Hz.

1.1.5. To record and statistically remove artifacts associated with eye movements, additionally record electrooculograms. Select the target electrodes based on theoretical interests. In addition, use individual MRI and computed tomography data to check the electrode locations.

1.2. Sample and preprocess trial intracranial EEG data (**Figure 1**).

Note: The analytical protocols can be applied to various types of data without any restriction to specific data-length or preprocessing methods.

1.2.1. Here, sample data during 3000 ms (pre-stimulus: 1000 ms; post-stimulus: 2000 ms) for each trial.

1.2.2. Because participants here showed abnormally high amplitude activity in some trials, possibly related to epilepsy, exclude these outlier trials using predefined thresholds (> 800 μV

and > 5 *SD*). Other preprocessing steps, including visual inspection and independent component analysis, may be required depending on the experimental objectives and conditions.

2. Time–Frequency SPM Analysis

2.1. Set up SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) and use the M/EEG analytical menu[13] (**Figure 2**).

2.2. Perform the time–frequency SPM analysis[2] by selecting **Time–frequency analysis** in the SPM menu for the preprocessed intracranial EEG data of each trial using continuous wavelet decomposition with Morlet wavelets based on predefined parameters (**Figure 3**).

Note: Wavelet transforms reveal the temporal evolution of spectral components by convolving intracranial EEG data with wavelets of multiple frequencies[14].

2.2.1. Here, conduct wavelet decomposition using seven-cycle Morlet wavelets for the entire epoch (-1000–2000 ms) and frequency range of 4–300 Hz.

2.2.2. Determine the mother wavelet and number of cycles based on a previous study[15]. Note that the number of cycles in the wavelet controls the time–frequency resolutions and is recommended to be greater than 5 to ensure estimation stability[13].

2.2.3. Determine time and frequency ranges based on the research interest.

2.3. Crop the resultant time–frequency maps automatically by selecting **Crop** in the SPM menu to remove edge effects. Here, crop the time–frequency maps into -200–500 ms.

2.4. Perform the data transformation (optional) and baseline correction by selecting **Time-frequency rescale** in the SPM menu for the time–frequency maps to visualize the event-related power changes better and improve the normality of the data.

Note: Here, the time–frequency maps were log-transformed and baseline (-200–0 ms)-corrected.

2.5. Convert the time–frequency maps into two-dimensional (2D) images by selecting **Convert2Images** in the SPM menu.

2.5.1. Smooth using a Gaussian kernel with a predefined full-width half-maximum (FWHM) value to compensate for inter-subject variability and conform to the assumptions of the random field theory used in the statistical inference[2,13].

Note: Here, the time–frequency maps were smoothed with a Gaussian kernel of FWHM of 96 ms in the time domain and 12 Hz in the frequency domain based on a previous study[2].

2.6. Enter the 2D images into the general linear model by selecting **Specify 1st-level** in the SPM menu.

2.7. Estimate the general linear model by selecting **Model estimation** in the SPM menu.

2.8. Perform statistical inferences on the time–frequency SPM{*T*} data based on the random field theory[2] by selecting **Results** in the SPM menu. Detect significantly activated time–frequency clusters with predefined thresholds (possibly corrected for multiple comparisons).

Note: Here, the extent threshold of $p < 0.05$, which was FWE-corrected for multiple comparisons, with a height threshold of $p < 0.001$ (uncorrected) was used.

3. DCM of Induced Responses

3.1. Set up SPM12 (http://www.fil.ion.ucl.ac.uk/spm/) and use the M/EEG analytical menu[13] (**Figure 4**).

3.1.1. Start DCM analysis by clicking **DCM** button in the SPM menu. Activate DCM for induced responses by selecting **IND** in the list box. Import the preprocessed intracranial EEG data by clicking **new data** in the DCM for M/EEG menu.

3.2. Specify time window of interest, conditions of interest, contrasts for the selected conditions (this define the modulation inputs later used in network specification), frequency window of interest, and the number of the wavelet cycles in the DCM for M/EEG menu (**Figure 5**).

3.2.1. Use five-cycle Morlet wavelets (4–100 Hz in 1-Hz steps) and set the time window to 1–500 ms.

3.2.2. Determine the wavelet cycle in accordance with the default setting. Note that the software recommendation is the value greater than 5 to ensure estimation stability[13]. The time–frequency ranges were determined based on our research interest. Note that a time window with an additional ± 512 ms was automatically used during computation to remove edge effects.

3.3. Based on the DCM framework[4,5], define the (1) driving inputs, which represent the sensory inputs on neural states; (2) intrinsic connections, which embody the baseline connectivity among neural states and self-connections; and (3) modulatory effects on intrinsic connections *via* experimental manipulations for null and hypothesized models. Also define the type of modulation as linear (within-frequency) or nonlinear (between-frequency).

3.3.1. Specify intrinsic (linear and nonlinear) connections, driving inputs, and modulation inputs in the DCM for M/EEG menu. Select **Frequency models** in the list box to save frequency–frequency modulatory coupling parameter images.

3.3.2. Modify the default settings of some related parameters (*e.g.,* prior stimulus onset time and duration) if necessary. Estimate the models by selecting **invert DCM** in the DCM for M/EEG menu.

3.4. Conduct a random-effects Bayesian model selection (BMS) analysis[17] by clicking **BMS** in the DCM for M/EEG menu to identify the optimal network model. Use the model expected probabilities and/or exceedance probabilities as evaluation measures.

3.5. Make inferences regarding the cross-frequency patterns of the modulatory connections using the winning model parameters by using the SPM menu (see Step 2).

3.5.1. Smooth the modulatory coupling parameter images by selecting **Convert2Images** in the SPM menu.

3.5.2. Perform general linear model analyses by selecting **Specify 1st-level** in the SPM menu.

3.5.3. Calculate the 2D SPM{*T*} values by selecting **Results** in the SPM menu.

Note: Here, the FWHM was set at 8 Hz based on a previous study[4]. Significant values were exploratorily identified using a height threshold of $p < 0.05$ (uncorrected).

REPRESENTATIVE RESULTS

Using the protocol presented herein, we analyzed intracranial EEG data in response to faces[18,19]. We recorded data from six patients during the passive viewing of faces, mosaics, and houses in upright and inverted orientations. The contrasts of upright faces *versus* upright mosaics and upright faces *versus* upright houses revealed the face effect (*i.e.,* face-specific brain activity relative to other objects). The contrast of upright faces *versus* inverted faces revealed the face-inversion effect (*i.e*., face-specific visual processing possibly related to configural/holistic processing[20]). As the target region for the time–frequency analysis and phase–amplitude cross-frequency coupling, we selected the right inferior occipital gyrus (IOG) based on previous neuropsychological[21] and neuroimaging[22] findings. For DCM, we tested the model in which the IOG and amygdala constitute a functional network during face processing based on previous anatomical evidence[23].

Time–frequency analysis

Time–frequency analyses were conducted to investigate the temporal and frequency profiles of IOG activity during the processing of faces. Time–frequency maps were converted into 2D images and entered into the general linear model with the factors of stimulus type (face, house, and mosaic) and orientation (upright and inverted). Significant responses were identified using an FWE-corrected extent threshold of $p < 0.05$ with a height threshold of $p < 0.001$ (uncorrected). The contrasts testing the face effect (upright face *vs.* upright mosaic) consistently revealed significant rapid (110–500 ms) gamma-band activity (**Figure 6a**). The

contrasts testing the face-inversion effect (upright face *vs.* inverted face) revealed significant gamma band activity at a later period (195–500 ms).

DCM of induced responses

DCM of induced responses was applied to test the functional network models of the IOG and amygdala. For both the face and face-inversion effects, the exceedance probabilities of the random-effects Bayesian model selection indicated that the model with intra-regional modulatory connectivity in both regions and bidirectional inter-regional modulatory connectivity was the most likely among all possible models (**Figure 6b**).

Next, we inspected the spectral profiles of the modulatory couplings in the best model. Significant effects were assessed for the entire spectral range with a height threshold of $p < 0.05$ (uncorrected). Significant same- and cross-frequency modulatory couplings were observed for both intra- and inter-regional connectivity of both the face and face-inversion effects (**Figure 6c**). For example, as the intra-regional modulation of the face effect, the amygdala showed negative gamma–gamma same- and beta–gamma cross-frequency couplings. Meanwhile, the intra-IOG modulation showed a positive cross-frequency coupling in the theta/alpha/beta–gamma band. In addition, as the inter-regional coupling of the face effect, the IOG–>amygdala modulation revealed a profile in which the theta/alpha band in the IOG facilitated the gamma band in the amygdala. For amygdala–>IOG modulation, the gamma band in the amygdala inhibited the theta/alpha band and the same-frequency gamma band in the IOG. For the face-inversion effect, similar amygdala–>IOG modulation, in which the gamma band in the amygdala inhibited the gamma band in the IOG, was observed. However, for IOG–>amygdala modulation, the theta/alpha–gamma association observed in the face effect was not evident.

FIGURE AND TABLE LEGENDS

Figure 1. Illustration of the generation of the trial intracranial electroencephalography data.

Figure 2. Graphic user interfaces of the Statistical Parametric Mapping (SPM) software for time–frequency analyses. (1) SPM menu. **(2)** Time–frequency analysis. **(3)** Cropping. **(4)** Baseline correction. **(5)** Conversion. **(6)** Statistical model. **(7)** Model estimation. **(8)** Statistical inference.

Figure 3. Flowchart of the time–frequency analysis using the Statistical Parametric Mapping (SPM) software. (a) Prepare the preprocessed intracranial electroencephalography (EEG) data of each trial. **(b)** Conduct time–frequency (TF) decomposition for the EEG data using continuous wavelet transform. **(c)** Crop, log-transform, and baseline correct for the TF maps. **(d)** Convert the TF maps into two-dimensional (2D) images. **(e)** Enter the 2D images into the general linear model. **(f)** Perform statistical inferences on the TF SPM{*T*} data.

Figure 4. Graphic user interfaces of the Statistical Parametric Mapping (SPM) software for dynamic causal modeling (DCM) analyses. (1) SPM menu. **(2)** DCM menu. **(3)** Bayesian model selection. **(4)** Smoothing. **(5)** Statistical model. **(6)** Model estimation. **(7)** Statistical inference.

Figure 5. Flowchart of the dynamic causal modeling of induced responses. (a) Calculate the time–frequency spectra for each trial of the targeted multiple electrodes (the inferior occipital gyrus (IOG) and amygdala in our example) using continuous wavelet decomposition. Average the spectral magnitudes of time-frequency responses to yield the induced response. Then, define the driving input, intrinsic connections, and modulation of intrinsic connections by experimental manipulations. Construct models to test hypotheses and estimate the models. **(b)** Conduct a random-effects Bayesian model selection analysis to identify the optimal model. **(c)** Convert the frequency–frequency modulatory coupling parameters into two-dimensional (2D) images (with smoothing). Then, perform random-effects general linear model analyses and calculate the 2D SPM{*T*} values.

Figure 6. Representative results. (a) Time–frequency maps of right inferior occipital gyrus (IOG) activity for the upright face (FU; left) and upright mosaic (MU; middle) conditions. The SPM{*T*} data for FU *versus* MU are also shown (right). **(b)** Functional network models in the IOG and amygdala. Eight possible combinations of modulatory input by FU *versus* MU onto connections between the IOG and amygdala and self-connection onto each region were investigated. **(c)** Frequency–frequency modulatory coupling parameters and SPM{*T*} values for FU *versus* MU of IOG–>amygdala and amygdala–>IOG modulation are shown. The red–yellow and blue–cyan blobs indicate significant positive/excitatory and negative/inhibitory connectivity, respectively.

DISCUSSION:

The analytical protocols for intracranial EEG data using the SPM software introduced herein have several advantages compared with functional MRI. First, the protocols can depict neural activation at a high temporal resolution. Therefore, the results indicate whether the cognitive correlates of neural activation are implemented at early or late stages of processing. In our example, the face effect was identified during the very early stages (*i.e.,* 110 ms) of visual processing. In addition, the comparison of the temporal profiles of neural activity related to different psychological functions provide interesting implications. In particular, our example revealed different activation times for the face and face-inversion effects, beginning at 115 ms and 165 ms, respectively, in the IOG. Such rich temporal information can deepen our understanding of neurocognitive mechanisms.

Furthermore, the protocols can depict intra- and inter-regional neural connectivity. Data from other neuroscientific measures, such as hemodynamic signals and scalp-recorded electromagnetic signals, contain a large amount of noise and require estimation based on several assumptions to extract the original electric signals, which can distort the resultant neural connectivity. Hence, the analysis of directly recorded electric signals is valuable. As an illustration, although our results revealed functional coupling between the IOG and amygdala during face processing, such coupling was not detected in a previous analysis of functional MRI data[24]. Understanding neural mechanisms requires the identification of causal relationships among neural circuits, which requires temporal information of neural activation.

However, it is important to note that the optimal protocol used to record and analyze intracranial EEG remains debated. For example, methodological studies have suggested that

reference electrodes can pick up bodily physiological artifacts (*e.g.*, eye movements and muscle activity) and environmental noise, and the suitable position of reference electrodes for intracranial EEG remains to be determined[25,26]. Several preprocessing methods (*e.g.*, filtering and non-linear transformations) to remove artifacts (*e.g.*, epileptic activity) have been proposed, although they are under debate[27]. A study also reported that time–frequency analyses using wavelet decomposition could blur the peaks in the original data[28], and alternative analytical methods, such as the Hilbert–Huang transform, may offer better temporal resolutions[29]. The extraction of the high-frequency range may also be improved using such methods[30]. It has been noted that cross-frequency coupling could be biased by sharp non-linear transients and controlling for such confounding effects is needed[31–33]. Several different analytical methods have been proposed for the analysis of intra- and inter-regional coupling, such as the phase-locking value[16], weighted phase lag index[34], and Grander causality[35], and it remains unclear which analyses and parameters (*e.g.*, frequency) are the most relevant to cognitive processing[3]. In some cases, intracranial EEG data may not satisfy parametric assumptions and non-parametric analyses may be optimal[36]. Recently, other analytical protocols have been proposed[37]; compared with other protocols, those based on the SPM software may have the unique potential to provide a unified framework for the analysis of various types of neuroscientific data[38]. Researchers should pay close attention to advancements in analytical protocols for intracranial EEG data.

In summary, we introduced analytical protocols that we recently applied to analyze intracranial EEG data, which include time–frequency SPM analysis, cross-frequency coupling, and DCM of induced responses. We believe these analytical protocols can identify neural correlates of cognitive functions with high spatial and temporal profiles.

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DISCLOSURES:

The authors have nothing to disclose.

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Figure 1

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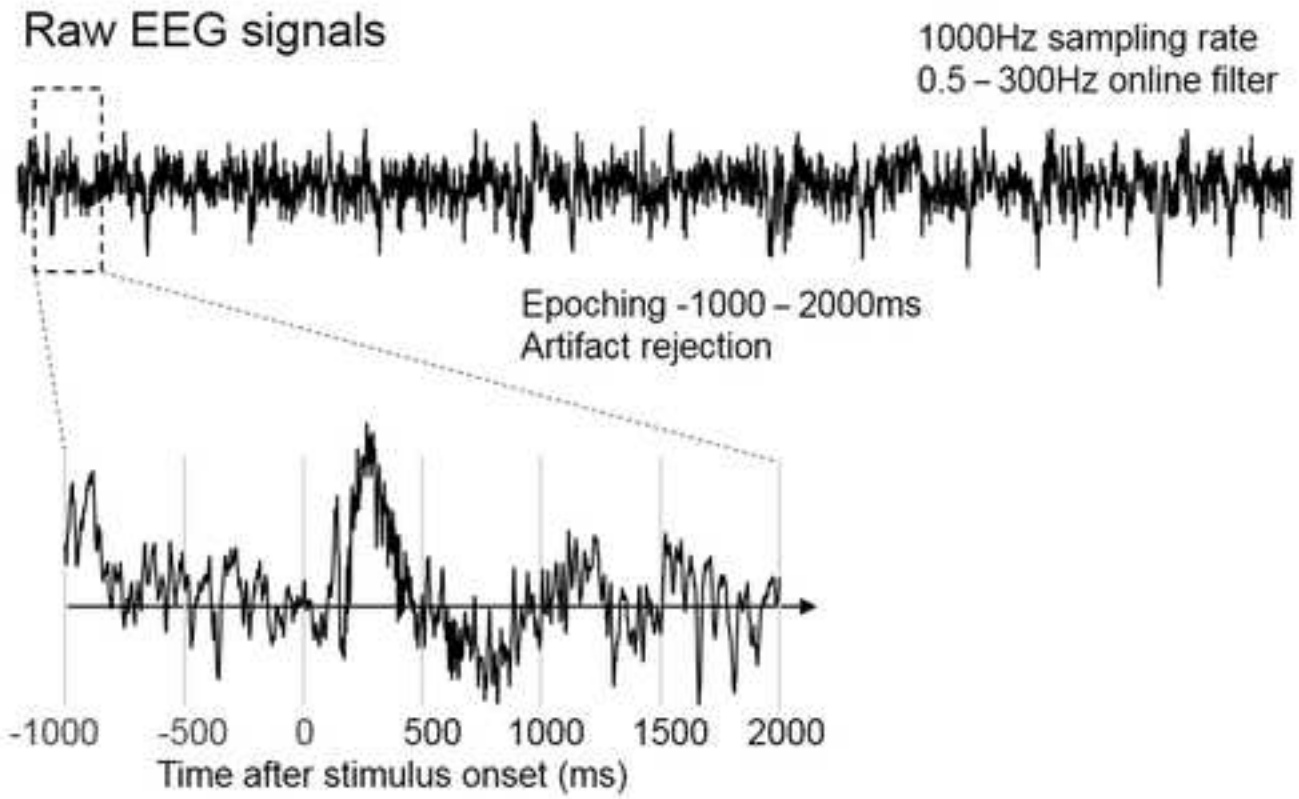

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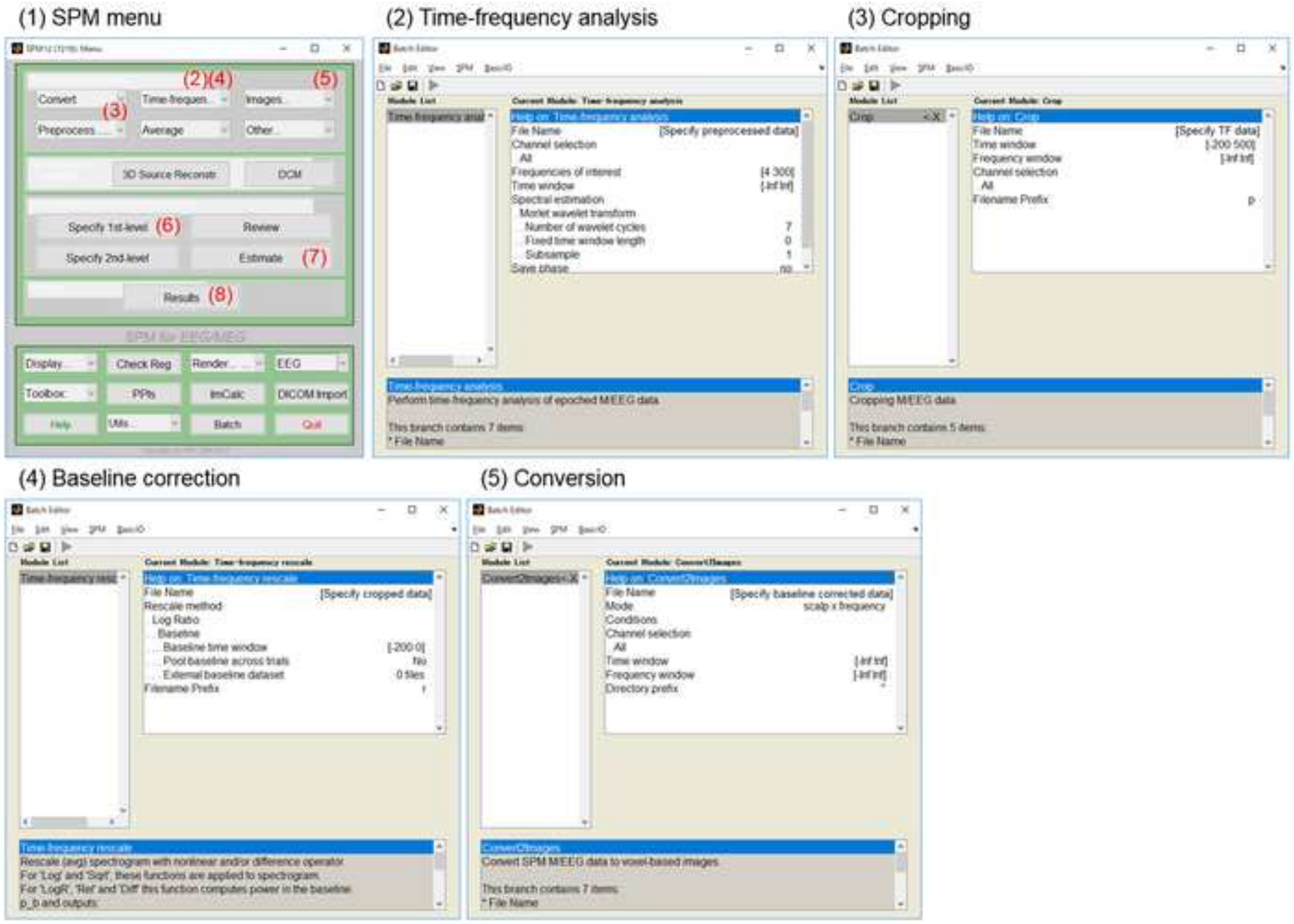

Figure 2 (2)

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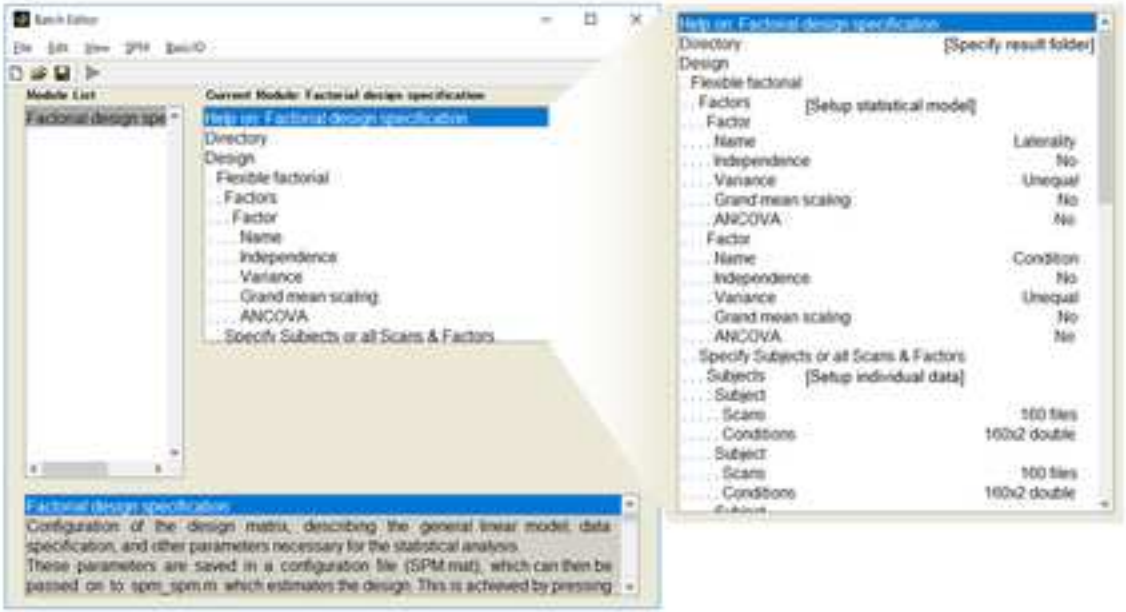
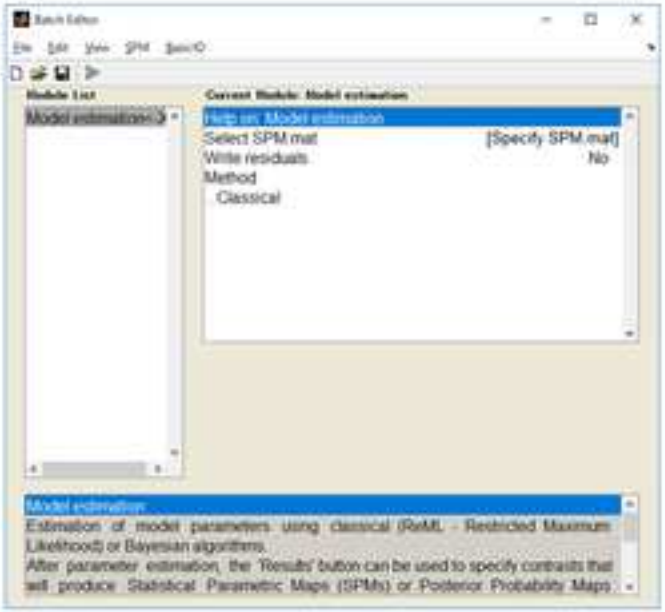
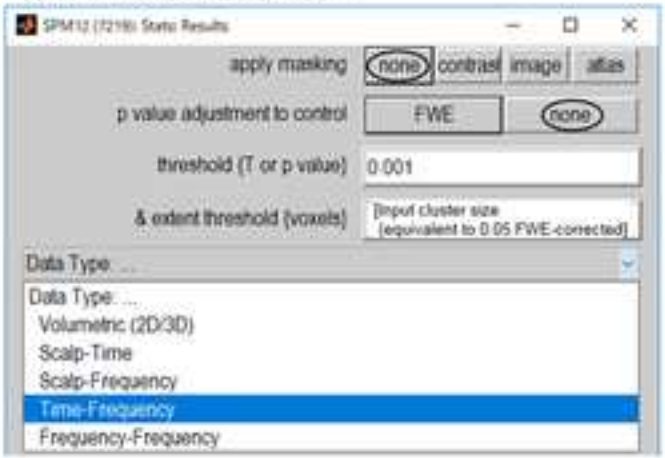
(6) Statistical model



(7) Model estimation



(8) Statistical inference



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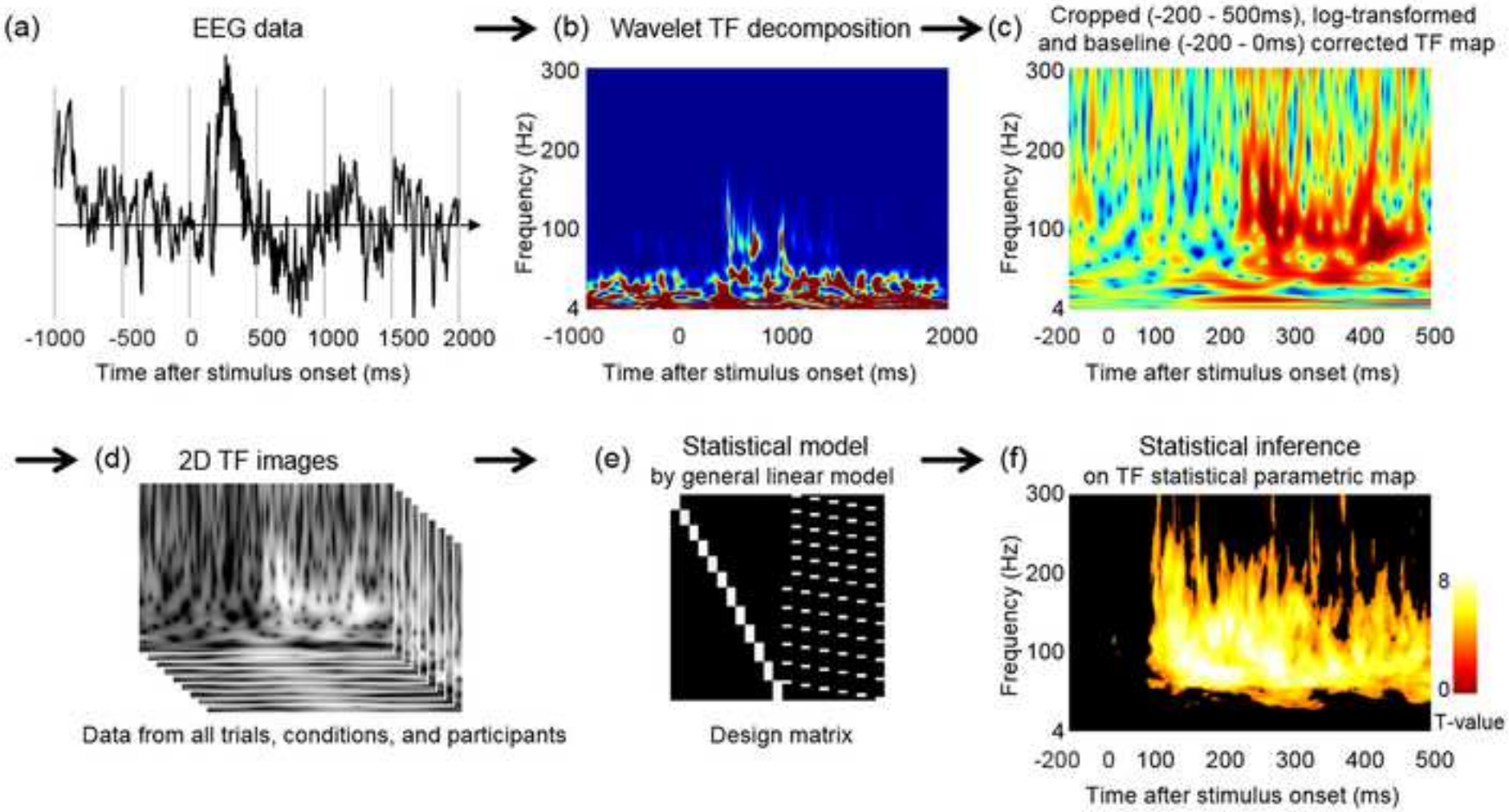

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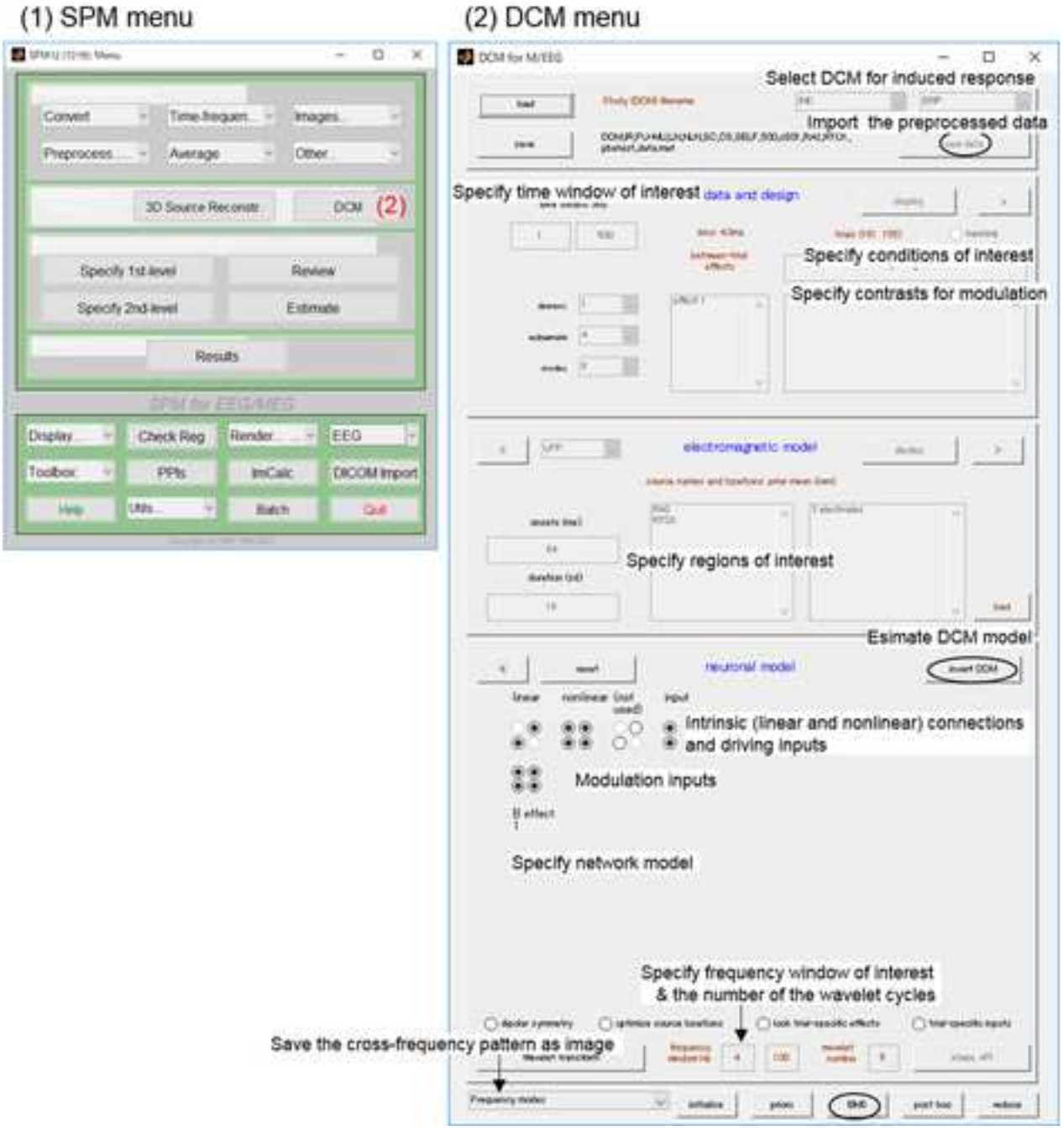

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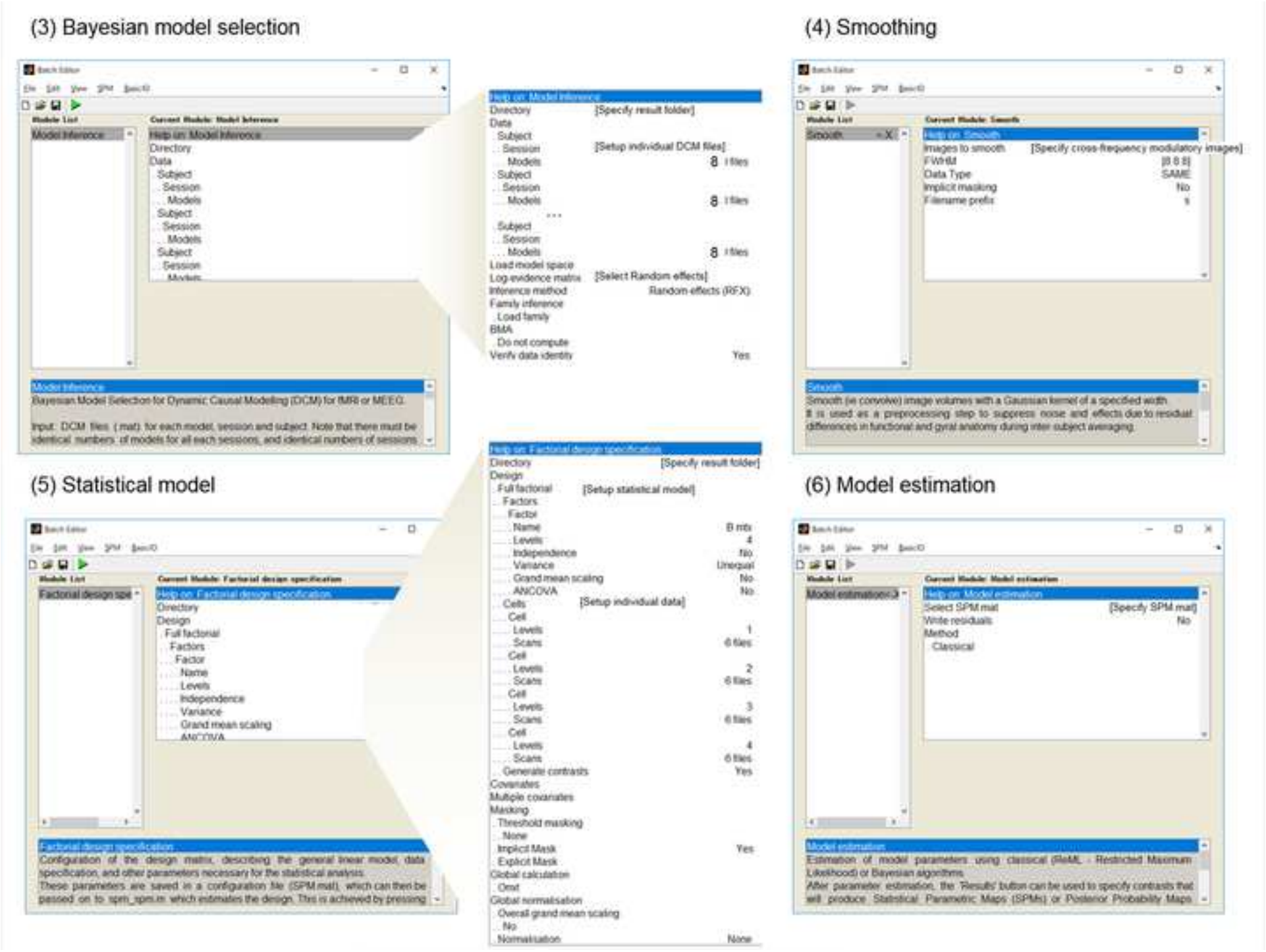

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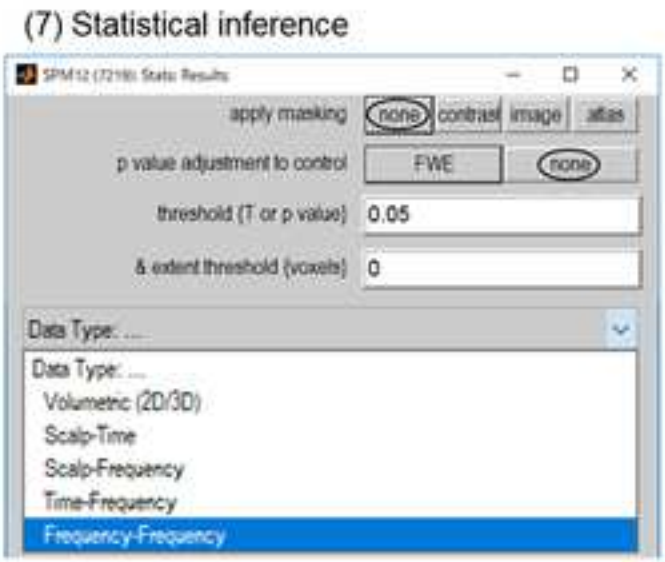

Figure 5

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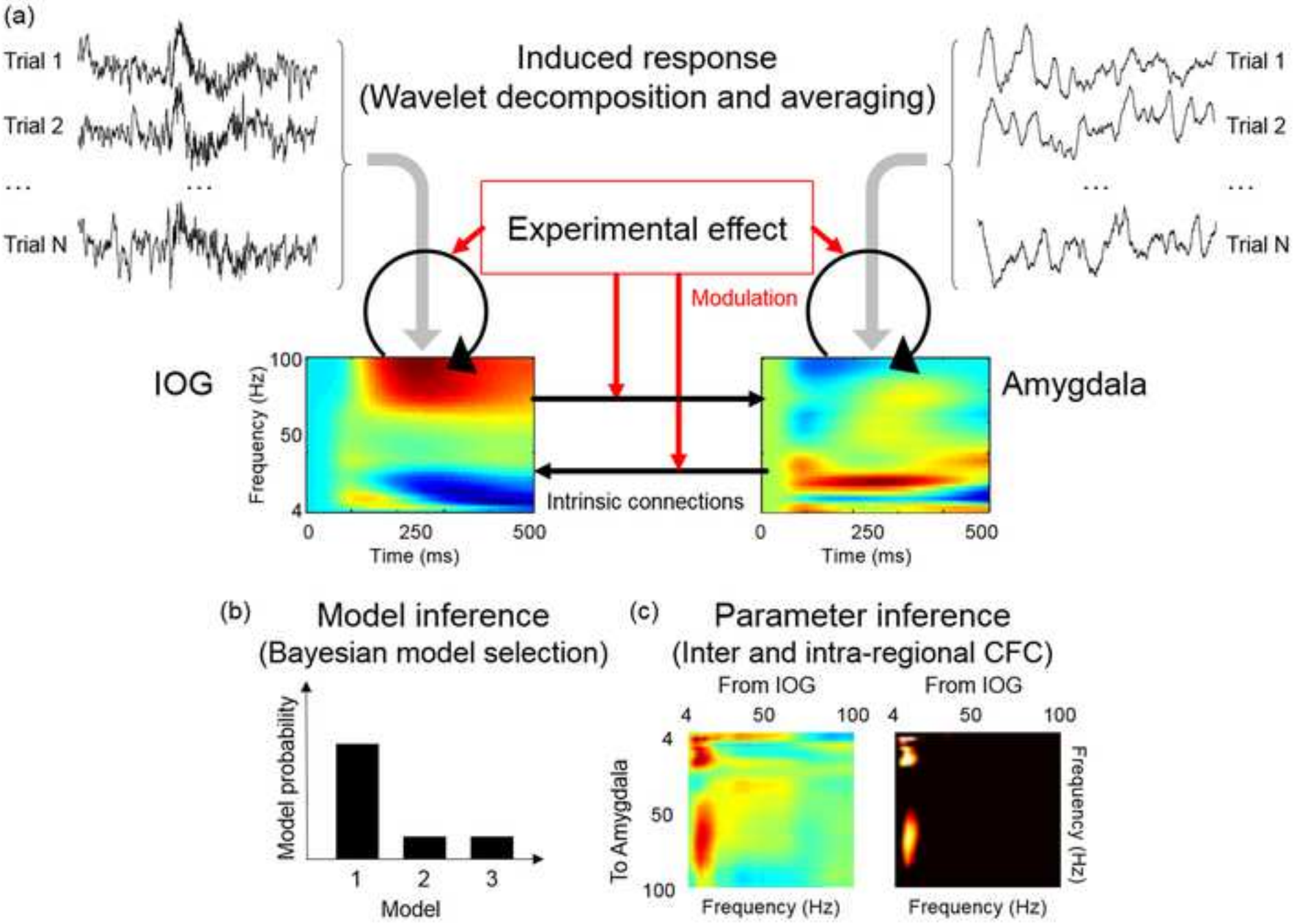

Figure 6

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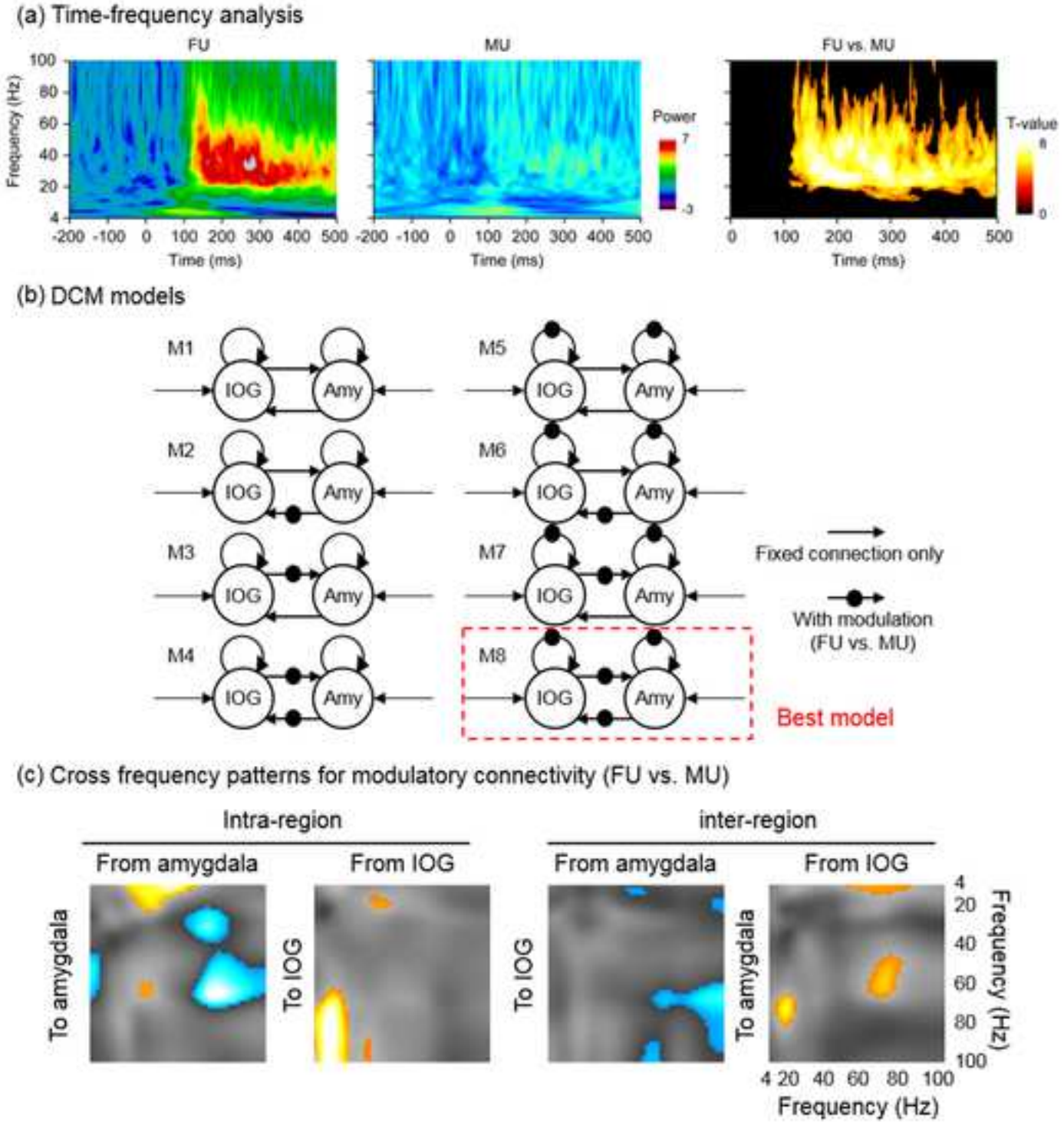

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Title of Article:	Analyzing neural activity and connectivity using intracranial EEG data
Author(s):	Wataru Sato, Takanori Kochiyama, Shota Uono, Naotaka Usui, Akihiko Kondo, Kazumi Matsuda, Keiko Usui, Motomi Toichi, and Yushi Inoue

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Title:	Dr
Signature:	Wataru Sato
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Rebuttal Letter
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Dear Professor Nguyen,

Thank you for asking us to revise our previously submitted manuscript (JoVE58187R3). We have made careful revisions to the manuscript based on the provided suggestions. Major changes to the manuscript are shown in red text. A professional English-language editing service made language-related changes, which are not highlighted unless the content was altered. We hope that the revised manuscript will be suitable for publication in *Journal of Visualized Experiments*.

Editorial comments

Point 1

Please take this opportunity to thoroughly proofread the manuscript to ensure that there are no spelling or grammar issues. The JoVE editor will not copy-edit your manuscript and any errors in the submitted revision may be present in the published version.

Response

As suggested, we have had the manuscript professionally proofread. For a certificate, please see:
http://www.textcheck.com/certificate/2bZ0RR

Point 2

Please provide the GUI instructions in the written manuscript as well. The script for the video is directly derived from the written protocol.

Response

As suggested, we have added the GUI instructions in the main text of the revised manuscript.

Point 3

Please add more details to your protocol steps. Please ensure you answer the "how" question, i.e., how is the step performed? Alternatively, add references to published material specifying how to perform the protocol action.

Response

As suggested, we have added a more detailed description (texts and figures) of the protocol.

Point 4

1.1: How were the EEG data recorded?

1.1.1: Please include how the electrodes were implanted here instead of referencing the details out.

Response

We are unable to provide video data for recording intracranial EEG, because they contain clinical and personal information. Therefore, we focused on the data analytical protocol in this article. Because recording and referencing methods differ across institutions and our analytical protocols can be applied to various types of data, we introduced our recording and referencing method as an example. We would prefer not to modify the Basic Information section.

Point 6

How many patients are there? What are the inclusion/exclusion criteria?

Response

As suggested, we have added these details (p. 5).

Point 6

For steps 2-4, please specify the click by click instructions to perform the protocol with the GUI.

Response

As suggested, we have described the click-by-click instructionsin the revised manuscript. Because the phase–amplitude cross-frequency coupling analysis reported in the previous manuscript required code (CUI operations), we have removed this analysis from the revised version.

Reviewer #1

Point 1

What code was used, if any, to run this analysis. Code snippets should be presented, perhaps in a supplement or video.

Response

Because the editor discouraged the description of protocols requiring code, we have described only protocols for analysis using the SPM software, which did not require code.

Point 2

Many parameter choices were made in each analysis; justification or explanation for parameter and/or window size choice is needed.

Response

As suggested, we have added descriptions and justifications for the parameters used in our research. These parameters were generally selected from previous studies.

Point 3

The authors mention that other iEEG pipelines exist - what is the advantage of this pipeline versus other iEEG pipelines? Brief explanations would be appreciated.

Response

We have added a description of the potential advantages of the proposed analytical protocol to the Discussion (p. 14). Analytical protocols that use SPM can provide a unified framework for the analysis of various types of neuroscientific data.

Point 4

A description of each technique (e.g. DCM or phase amplitude coupling) in "plain english" so that the reader knows what is being measured or estimated. For example, what measure of connectivity is used in DCM?

Response

As suggested, we have added a description of what is being estimated to the Introduction (p. 4). The time–frequency SPM analysis reveals the time and frequency at which brain regions are activated and DCM analysis shows how brain regions communicate.

Point 5

Add statistics to the results section so that the reader can get a sense of what effect sizes can be expected.

Response

As suggested, we have added the statistical thresholds for our representative results in the revised manuscript (pp. 11–12).

Point 6

The authors mention using both cortical surface and depth electrodes. Are data from these two electrode types treated the same? Are they recorded simultaneously? Is the same reference used?

Response

As suggested, we have added information on the electrodes, recording, and reference for our study in the revised manuscript (p. 5). Recordings by surface and depth electrodes were made simultaneously using the same reference, and the data from both sources were analyzed in the same manner.

Point 7

It would be nicer to provide the pros and cons of the reference chosen in this study. In our opinion, such a reference electrode would pick up a lot of environmental noise/EMG artifacts and thus potentially be a problematic reference. For example, the amygdala is quite proximal to the ocular and temporal muscles. Without using a more suitable reference, an amygdala channel would suffer from EMG artifacts related to saccades and overt responses (see: Prog Neurobiol. 2012;98:265-78; Brain Topogr 2009;22:18-23).

Response

As suggested, we have added a description of the possible artifact contamination of reference electrodes to the Discussion section, as an unresolved issue (p. 13). We have also added our rationale for the current reference electrode position by citing the methods of a previous methodological study in the Protocol section (p. 5).

Reviewer #2

Point 1

How to deal with epileptic activity.

Response

As suggested, we have added a description of our methods for reducing the effect of epileptic activity to the Protocol section (p. 6). We excluded outlier trials based on predefined thresholds. Because the editor requested that we describe protocols that can be visualized using GUIs, and this operation cannot be visualized, we briefly described our approach as an example of an appropriate method.

Point 2

How to reconstruct the exact positions of electrodes

Response

We used individual MRI and CT datasets to verify the electrode locations. We have added this information to the Protocol section (p. 6). However, because the editor requested that we describe only protocols that can be visualized, and videos of clinical operations, including CT image acquisition, cannot be shared, we briefly described approach as an example of an appropriate method.

Point 3

The effects and choice of how to reference and preprocess the data

Point 4

The effects of filtering on epileptic or sharp-transient, non-sinusoidal activity

Response

As suggested, we have added a discussion of the influences of preprocessing, filtering, and the reference to the Discussion as

unresolved issues (p. 13).

Point 5

Provide insights into how time-frequency analyses work and how one is supposed to design them given that short transients and epileptic or oscillatory bursts can easily be blurred in the frequency domain by adjusting the settings. I believe that the use of a GUI does not facilitate that level of understanding that is necessary to characterize iEEG data.

Response

As suggested, we have described the principle behind time–frequency analyses in the Protocol section (p. 7). We agree that the time–frequency analyses introduced in this study could blur peaks in the original data, and that it is difficult to compensate for this blurring by adjusting the settings. Therefore, we have added a discussion of this drawback to the Discussion section (p. 13).

We have described our GUI-based protocols in this study because the editor encouraged us to present GUI-based protocols. We believe that the GUI illustrations will help readers to understand how to use our protocols.

Point 6

The use of multiple toolboxes and the effect of manual 'cropping' likely does not lead to better scientific and statistical practice.

Response

As suggested, we have focused on a single toolbox (SMP) in this article. Because we conducted automatized cropping in all trials, we have described this procedure in the Protocol section in the revised

manuscript (p. 7).

Point 7

No justification of the employed stats. E.g. using a GLM in SPM might be applicable in fMRI in certain instances, but the non-linear nature of iEEG is better suited by e.g. the approach by Maris and Oostenveld (2007)

Response

As suggested, we have added a discussion of the possible suitability of nonparametric analyses for intracranial EEG data to the Discussion (p. 14).

Point 8

Extraction of HFB activity is non-trivial, simply band-pass filtering with a e.g. 70-150 Hz filter includes a sharp drop-off in the 1/f spectrum and hence the authors need to discuss alternative approaches as e.g. used by Forster (2015) or Holdgraf (2016)

Response

As suggested, we have discussed alternative approaches to the extraction of HFB activity in the revised Discussion (p. 14).

Point 9

No critical discussion of Cross-Frequency Coupling and in particular the 'Event-related' approach, which is highly susceptible to artifacts by non-linear sharp transients as e.g. outlined in Aru (2015), Gerber (2016) or Cole and Voytek (2017)

Point 10

The exact same holds true for their use of DCM

Response

In the revised manuscript, we have cited these reports in the Discussion to show that cross-frequency coupling could be biased by sharp non-linear transients and that control for such confounding effects may be necessary (p. 14).

Point 11

Critical discussions and methods considerations are missing in terms of cross-frequency coupling and connectivity metrics (e.g. coherence and the plv, considerations on volume spread, the effects of differences in trial numbers and differences in oscillatory power and how to account for them, e.g. pairwise phase consistency metrics or weighted phase-lag index)

Response

As suggested, we have explained in the revised Discussion that several different approaches have been proposed for the analysis of neural coupling, and that it remains unclear which analyses and parameters might be best suited for cognitive processing (p. 14).

Point 12

No information on assessing directionality by e.g. Granger or the phase-slope index. See e.g. Zheng et al. (2017) Nature Communications for a successful example on how to address some of these issues

Response

As suggested, we have mentioned in the revised Discussion that Granger causality could be an important alternative (p. 14).

Point 13

Essential citations on e.g. CFC are missing (that by the way published the code along with the paper to facilitate replicability: e.g. Canolty 2006 Science, Voytek 2010 Frontiers, Tort 2008)

Response

As suggested, we have cited these important studies in the revised manuscript (p. 4).

Point 14

The authors might want to consult e.g. Stolk (2018) Nature Protocols for more state-of-the-art tutorial.

Pesaran (2018) Nat Neurosci

Parvizi and Kastner (2018) Nat Neuro published authoritative guidelines on this topic.

In its present form and its gui-based approach this tutorial could potentially do more harm than advance the field because users will unlikely develop a deeper understanding on how these metrics should be used and the analyses should be carried out. While the field needs better tutorials, this manuscript one falls very short and hence should not published in its present form. Open access tutorials like the manuscript by Stolk (2018, also on biorxiv) and the freely available fieldtrip toolbox http://www.fieldtriptoolbox.org/tutorial provide more information.

Response

We appreciate the suggestion of these relevant studies. We have cited these studies in the revised Introduction (p. 5) and Discussion (p. 14). We have also explained in the revised Discussion that the protocols that used the SPM software may have the unique potential to provide

a unified framework for the analysis of various types of neuroscientific data (p. 14).

The editor encouraged us to provide a GUI-based approach to avoid describing abstract concepts in the Protocol section. We believe that our manuscript will be helpful to readers who wish to understand how to analyze intracranial EEG data using the SPM software.

All comments provided were helpful in the process of revising our manuscript. We hope that the revised manuscript has been sufficiently improved for publication in *Journal of Visualized Experiments*. Thank you for your interest and support.

Yours sincerely,

Wataru Sato