SoftwareX 11 (2020) 100434

Contents lists available at ScienceDirect

SoftwareX

journal homepage: www.elsevier.com/locate/softx

Original software publication

UF^{2}C — User-Friendly Functional Connectivity: A neuroimaging toolbox for fMRI processing and analyses

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ARTICLE INFO

Article history:
Received 16 April 2019
Received in revised form 16 December 2019
Accepted 25 February 2020

Keywords:
EEG-fMRI
Functional-connectivity
fMRI block-design
Event-related analysis

ABSTRACT

The User-Friendly Functional Connectivity (UF^{2}C) software provides researchers with a platform to analyze functional magnetic resonance neuroimages from the initial preprocessing steps to the generation of manuscript-quality figures. UF^{2}C is implemented in Matlab language and falls within the FreeBSD license. Our toolbox builds on a combination of existing methods to increase the user's power to perform different types of resting-state functional connectivity, event-related, and task activation analyses. To keep up with ongoing advances in this field, UF^{2}C is regularly updated to reflect these advances and incorporate user demands.

Code metadata

Current code version	*7.2 (Stable) 7.3 (Beta)*
Permanent link to code/repository used of this code version	https://github.com/ElsevierSoftwareX/SOFTX_2019_135
Legal Code License	FreeBSD license (BSD-2-Clause)
Code versioning system used	*none*
Software code languages, tools, and services used	*Matlab*
Compilation requirements, operating environments & dependencies	*Matlab (2012a or latter) and SPM12*
If available Link to developer documentation/manual	https://www.nitrc.org/docman/?group_id=1319
Support email for questions	brunno@fcm.unicamp.br

1. Motivation and significance

The use of magnetic resonance imaging (MRI) in neuroscience is fundamentally multidisciplinary, from instrumentation and development to clinical application. The concepts and theoretical basis of each MRI protocol demand an advanced understanding of physics and engineering, while their direct application requires additional statistical and clinical expertise for its accurate interpretation.

In a common MRI study, raw images are first preprocessed to remove potential artifacts and nuisance factors. Selecting which preprocessing steps to use from the large volume of existing options can be overwhelming, and not all of them are straightforward to use or fully implemented in the most popular neuroimaging toolboxes. Subject- and group-level analyses require additional selection of processing features, that are not straightforward to non-experts in the field. Even though the appropriate selection and its understanding are crucial for researchers to succeed, it can be discouraging for beginners and hard, if

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https://doi.org/10.1016/j.softx.2020.100434

not impractical, to perform large cohort studies when one lacks advanced coding skills.

The main motivation behind the development of UF^{2}C was to consolidate this diverse knowledge mentioned, streamlining the use of MRI in scientific investigations. UF^{2}C is designed to be user-friendly for the non-technical public. Its functionalities are the result of our combined experiences and extensive literature review which has led to the further optimization of presets. In other words, this software makes sophisticated image and signal processing methods accessible to researchers without a comprehensive background in MRI.

UF^{2}C has been employed by several researchers and students in centers across the world. Since 2016, in our center alone, the software was used and cited by 13 original research papers published in relevant journals (average JCR impact factor of 4.28). A list of these publications can be found in the **Supplemental Material**.

UF^{2}C runs within Matlab (The MathWorks, Inc) and incorporates open, third-party codes, mainly from Statistical Parametric Mapping 12 (SPM12) [1]. A full list of external files and functions are provided in the next section.

1.1. List of algorithms, tools, and software used

In addition to SPM12, UF^{2}C includes third-party open source algorithms in one or more functionalities (all license files accompany UF^{2}C installation files and are well credited):

- MANCOVAN: Copyright 2010, William Gruner
- FDR_BH: Copyright 2010, David Groppe
- Fillline: Copyright 2010, Shaz
- xticklabel_rotate: Copyright 2014, Brian Katz, Copyright 2009, The MathWorks, Inc
- multiWaitbar: Copyright 2016, The MathWorks, Inc.
- Neuromorphometric: Neuromorphometrics, Inc. (http://Neuromorphometrics.com/)
- uipickfiles: Copyright 2007, Douglas M. Schwarz
- BRAMILA tools: Enrico Glerean, Brain and Mind Lab at Aalto University

2. Software description

UF^{2}C was conceived for use in multidisciplinary laboratories, facilitating an intuitive and clean interaction with the user. In line with this main objective, the software offers many exclusive functionalities that aim to avoid biased group comparisons, and, consequently, misleading results (see Sections 3.1 and 3.1.5). With four functional connectivity analysis types and several combinations of preprocessing steps, UF^{2}C can accommodate most user demands, while respecting different theoretical approaches and providing capabilities comparable to other popular software [2]. For task-related protocols, our software offers a straightforward interface to design and run simple analyses for clinical research based on groups or individual subject analysis. Moreover, the *EEG-fMRI Analysis* (Section 3.2.3) encompasses a series of complex and laborious steps that have been carefully organized into a user-friendly interface. It is integrated with one of the most popular MRI-compatible EEG systems (Brain Products®) and guides the user from the initial steps through to the final outputs [3–5].

Each of the preprocessing and analysis steps contained within UF^{2}C has default values. However, all parameters and pipeline choices can be customized using the *uf2c_defaults* function. Users can easily modify these values through the graphical user interface (GUI) by clicking the "***Def***" button on the main software screen.

2.1. Software architecture

As UF^{2}C was initially created to complement SPM functionalities in Matlab, it is also implemented in Matlab language for maximum compatibility. It is designed to work with Windows, MacOS and Linux operating systems. In the neuroimaging field, fMRI protocols are broadly divided into two acquisition types: task (participants are required to perform a mental or physical task defined by the researcher) and resting-state (participants should not engage in any consistent or structured mental/physical activity). There are three basic domains in UF^{2}C: two types of fMRI analysis (task and resting-state) and one group of utility tools, all of which can be accessed via the GUI (Fig. 1) or via command line [6].

T*ask-related fMRI analysis* can be separated further into two categories: *block designs*, in which task and rest periods of 10 to 30 s (ideally) alternate predictably; and event-related designs when tasks are to be performed for short periods (2–6 s) at random[1] times during the scan. There is vast literature about experimental design itself of fMRI studies [7]. In our software, we consider epilepsy *EEG-fMRI studies* a specific, and more complex, case of event-related experimental design as the presence of interictal activity makes inclusion in the resting-state category inappropriate [4].

UF^{2}C offers an intuitive way to perform subject-level analyses on *task-related* and *EEG-fMRI event-related* designs.

The resting-state functional connectivity branch enables preprocessing, and subject- and group-level analyses, which are detailed in Section 3.1.

The *UF^{2}C tools* provide optional complementary functions that may be useful in common situations. A schematic of this structure can be found in Fig. 2.

2.2. Software functionalities

All functionalities are fully documented and explained in the software manual (https://www.nitrc.org/docman/?group_id=1319). The manual contains a complete overview of the techniques and accompanying theoretical basis of more complex methodologies. UF^{2}C currently has the following modules.

2.2.1. Modules for functional connectivity (resting-state)

- *Seed-based functional connectivity*
- *Functional Interactivity* — Automated multi-seed positioning
- *ROI Cross-Correlation* — Connectivity between coordinates or regions of interest (ROIs)
- *Cross-Correlation – Second Level* – Comparison between groups
- *Sliding Window Functional Connectivity*

2.2.2. Modules for functional MRI analysis (task and event-related)

- *fMRI Preprocessing*
- *First-Level Block Design Analysis*
- *EEG-fMRI Analysis*

2.2.3. UF^{2}C has currently the following tools

- *Movement Viewer*
- *Movement Analysis*
- *Outlier Detection*
- *Image Editor*
- *Filename Changer*
- *Tissue Segmentation*

[1] Random in this context means that task timing has no obvious structure, in contrast to the block design.

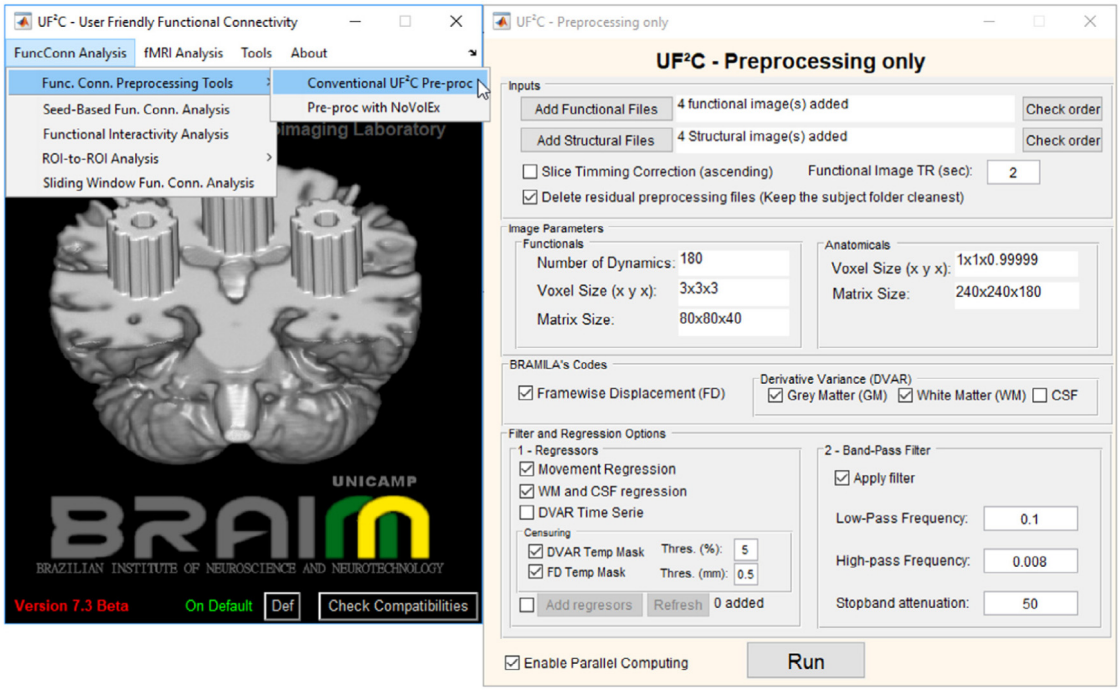

Fig. 1. The main screen and the *Functional Connectivity Conventional Preprocessing* windows of the GUI. All parameters are preset by the software and are based on a literature review or defined after experimental tests. In the window on the right, images from four subjects were selected and the GUI updates to inform the user about their choice.

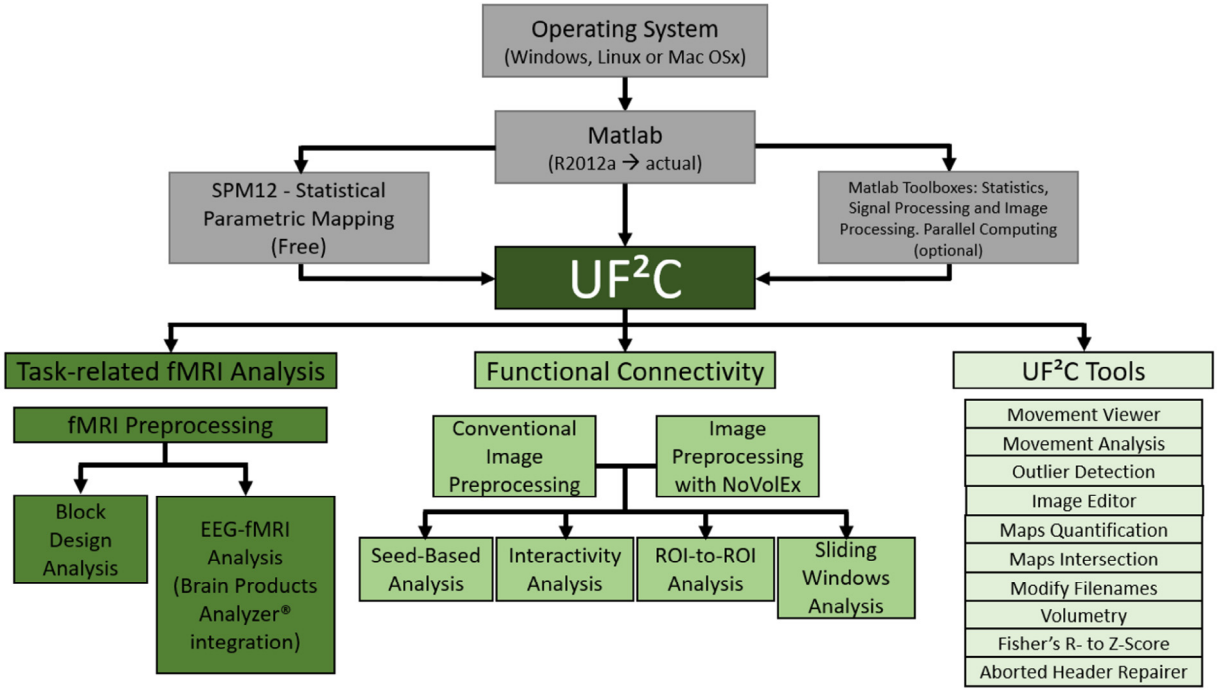

Fig. 2. Software architecture. In gray, all software requirements. In green, the software modules, and tools. A list of dependencies of each specific modality is found on the User Manual.. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

- *Volumetry* — calculates the volume (mm^3) of an ROI added or the intracranial volume
- *Interpolation tool* — convert image bounding box and voxel sizes to SPM8 default
- *R-score to z-score transformation (Fisher's)*: works with .mat files (vector, matrices...) and NIfTI files

3. Illustrative examples

3.1. Functional connectivity

In all four functional connectivity modules, the seed time-series extraction[2] respects the following criteria that improve the reliability of the connectivity resulting in a less biased group comparison:

A. Voxels within a seed are only included in the average time-series calculation if they are also within the subject's gray-matter (GM) mask;
B. UF^{2}C correlates each single seed voxel time-series with the average seed time-series (GM-masked). The voxel is only included (in the final average) if its correlation value is within the Quartile3–Quartile1 interval considering all correlations between the ROI-masked voxels (a stringent method for outlier definition).

This procedure is especially relevant when comparing subjects with structural brain alterations such as atrophies, focal cortical dysplasias or others, as it avoids the inclusion of voxels pertaining to the background, white-matter, or cerebrospinal fluid regions [7].

3.1.1. Functional connectivity — conventional preprocessing

The *Functional Connectivity Conventional Preprocessing* option reduces unwanted features/artifacts in the dataset without any statistical or connectivity inference. There are two different preprocessing options in UF^{2}C: conventional and NoVoLex. *Conventional* includes the most popular steps from SPM12: motion correction using a rigid body transformation; registration of the anatomical image onto the functional image; segmentation of white matter, gray matter and cerebrospinal fluid; normalization of the anatomical and functional images into the Montreal Neurological Institute brain template; and smoothing with a Gaussian kernel (full-width-at-half-maximum is two times the size of the voxel).

It should be noted that all UF^{2}C analysis modules are able to perform the preprocessing and subsequent connectivity analysis automatically.

Moreover, once you have the preprocessed functional images (FiltRegrSW_****.nii; obtained with this tool or with any other UF^{2}C Functional Connectivity module) you may skip preprocessing and quickly perform any connectivity analysis as preprocessing is often the most time-consuming step.

3.1.2. Functional connectivity preprocessing with NoVolEx

The NoVolEx (Noisy Volumes Exclusion) correction method was developed to exclude volumes with supra-threshold values of framewise displacement (FD) and/or derivative variance (DVAR) [8]. FD is a movement metric that condenses the six movement parameters determined during motion correction into one and gives an absolute measurement of head movement. DVAR is a metric based on voxel intensity and reflects how much the intensity changes in consecutive volumes. Changes that are too large, for instance, could be the result of head movement that caused background voxels (low intensity) to be shifted to the cortex (higher intensity).

To the best of our knowledge, to date, this tool is exclusive to UF^{2}C. It requires the definition of FD and DVAR thresholds as well as a set maximum number of volumes that may be removed. With these inputs, the routine will quantify the motion of all included images and use FD and DVAR to remove the supra-threshold volumes of each subject until the maximum number of deletions set by the user is reached. Further information can be found in the user manual.

[2] Seed time-series extraction is the process of averaging all time-series of the voxels contained in the seed.

3.1.3. Functional connectivity: seed-based analysis, first-level

The standard procedure for this type of analysis consists of two core steps: the extraction of an average time-series from a seed (violet cube in Fig. 3), and the calculation of the Pearson's correlation coefficient between the seed time-series and the time-series of all other voxels in the image, which is termed functional connectivity. This generates correlation maps like the one in Fig. 3, where large positive values are yellow (hot scale), and large negative values are cyan (cold scale). For group-level analysis, users are encouraged to transform correlation values into Fisher's-z maps which can be accomplished using the *Fisher's R- to Z-Score* UF^{2}C tool.

3.1.4. Functional connectivity: ROI-to-ROI analysis, first-level

In this analysis, time-series are extracted from all ROIs defined by the user and the Pearson's correlation coefficient is calculated between every ROI pair. This tool only performs the first-level analysis, that is, subjects are evaluated individually, and no group-level inference is performed. There are different ways to display the outputs of this module, the most common being in the form of adjacency matrices. Alternatives include more elaborated plottings like those shown in Fig. 4.

3.1.5. Functional connectivity: ROI-to-ROI analysis, second-level

From our perspective, any software that performs group comparisons as part of the ROI-to-ROI analysis should include comparisons of individual ROIs with group averages in opposite directions (negative correlation for a group and positive for another). Interpretation of correlation scores, which may be positive or negative, should not be purely mathematical. Correlation scores may be negative or positive, and the interpretations of these values should not be purely mathematical. Decreased connectivity and increased connectivity indicate lower or higher absolute Pearson's correlation scores comparing a group-A with a group-B. In this sense, the idea of "decreased" or "increased" means that the Person's correlation values are, respectively, farther or closer to zero when comparing two groups, regardless of whether the correlation is positive or negative. Interestingly, we can also detect some situations in which the correlation scores from a group-A and -B presents opposite signals. Despite the usage of absolute r-score values to define the direction of the differences among groups, UF^{2}C performs the statistical tests with the original values, making sure that comparisons among correlations in opposite directions have been considered accordingly in the statistical analysis. The main difference here is that for these scenarios, UF^{2}C graphical outputs will not show these alterations as decreased or increased, but will indicate them with dashed lines (on 2D plots) and yellow (on 3D plots) [2,9] (see Fig. 5).

3.2. Task-related fMRI analysis

3.2.1. Preprocessing

Similar to the Functional Connectivity Preprocessing, the *fMRI Preprocessing* functionality allows the user to prepare images for either Block Design (Section 3.2.2) or EEG-fMRI analysis (Section 3.2.3) by reducing the effect of unwanted artifacts. By default, UF^{2}C uses a traditional preprocessing pipeline. It should be noted that Slice Timing correction is disabled as activations elicited in block designs longer than 10 s with a TR less than 4 s are typically robust enough to exclude this step, preventing additional image interpolation [10]. Conversely, in event-related analyses, this step is normally included [10]. Using the GUI, users can specify which preprocessing steps they wish to include or exclude from the pipeline as well as select predetermined options for bounding box dimensions and voxel sizes.

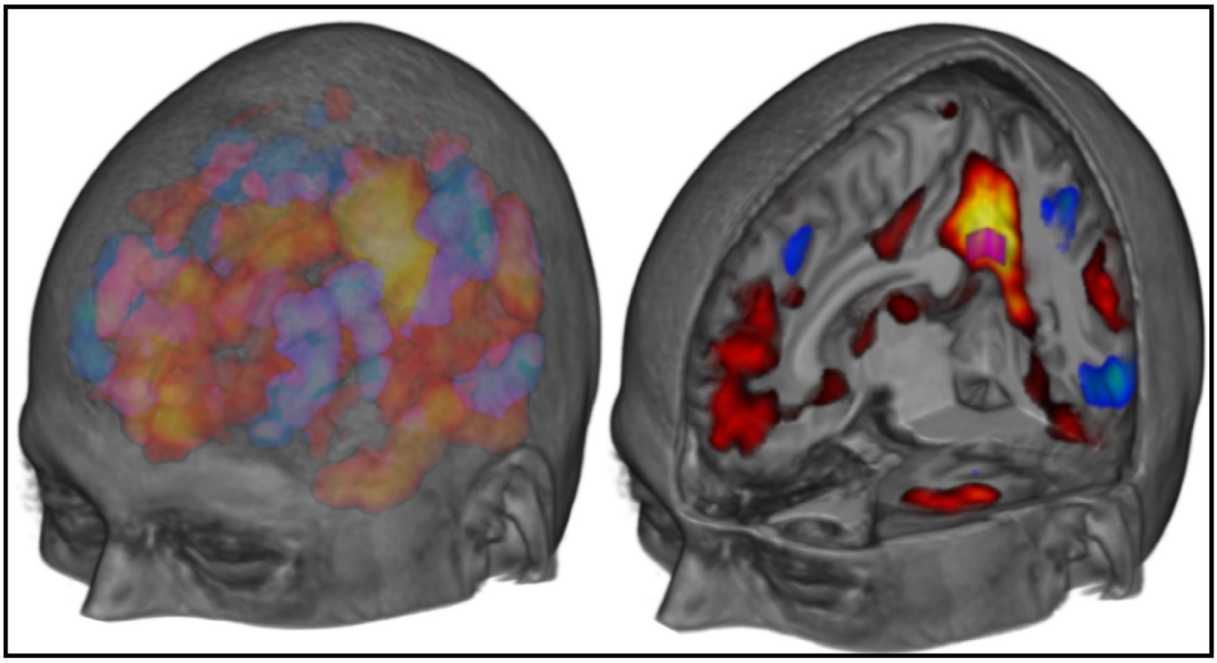

Fig. 3. Result example of an individual seed-based analysis. Rendering of the positive (hot scale) and negative (cold scale) connectivity maps related to a seed positioned in the posterior cingulate cortex (violet cube). The resulting maps in the figure were rendered using the MRIcroGL software (www.mccauslandcenter.sc.edu/mricrogl/home). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

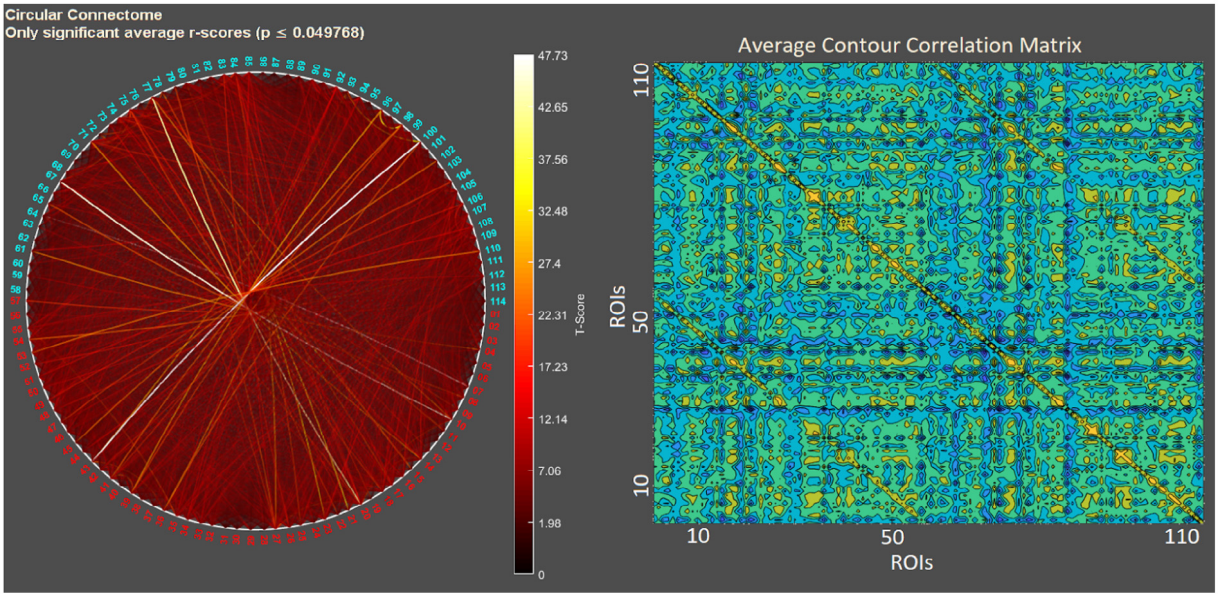

Fig. 4. Individual ROI-to-ROI analysis example result based on the correlation coefficient of 110 ROIs (55 left and 55 right hemisphere ROIs). On the left, the group average circular connectome represents all connections surpassing a significant t-score ($p<0.05$, r-score to t-score converted). On the right, the average correlation matrix with a topological grouping representation. A master file is saved for each group processed at the first level, and this file is used at the second-level to perform group inferences.

3.2.2. Block design analysis

First-Level Analysis (block design) has an intuitive interface with fields for the experimental parameters of the block design study. Upon populating these fields, users are able to: (i) plot their paradigm (rest and task blocks), (ii) plot a sample of the design matrix with the input parameters, (iii) define statistical thresholds for the first-level (subject level) analysis, and (iv) select parameters to generate figures of the results (see Fig. 6).

3.2.3. EEG-fMRI analysis

To perform event-related analyses, the timing of our events of interest are required. For example, by presenting a word to a participant for 2 s during the scan, we know at what times of the experiment stimulus and subsequent event of interest occurred. In an epilepsy study, however, we are interested in the timing of abnormal brain activity that occurs sporadically and unpredictably, and can only be determined by continuously recording EEG to capture these events. After denoising, the EEG data is read by a specialist who defines the timing of the events. The *EEG-fMRI*

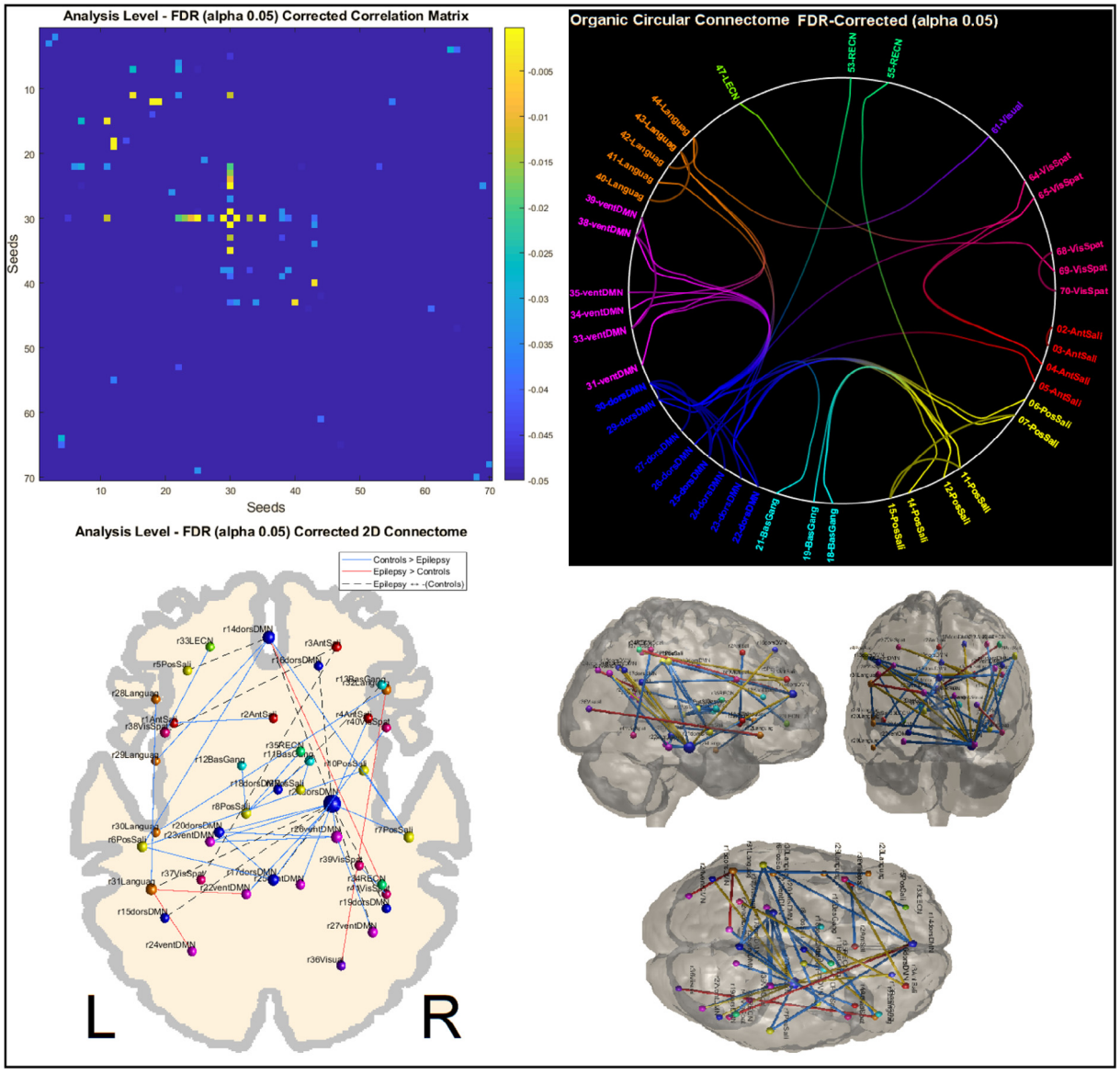

Fig. 5. Sample result from a group-level ROI-to-ROI analysis. The four images represent the same findings in distinct graphical representations of the nodes with significant difference ($p<0.05$, FDR corrected) between groups. On the top left, a correlation matrix view (adjacency matrix). In the top-right, the "organic" circular connectome with the lines color-coded by functional networks. On the bottom left, the 2D flat representation (axial plane) with the anatomical position of the included ROIs. On the bottom right, the 3D representation of altered connections with the respective position (centroid) of each ROI with altered connections. For the 2D and 3D graphics, the line colors indicate the direction of the alterations: Group-A>Group-B in blue, Group-B<Group-A in red, the dashed and yellow lines indicate signal in opposite directions between groups on 2D and 3D graphics respectively. The sphere colors represent the functional networks to which each ROI belongs [9] . (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Analysis can combine the EEG temporal information with the fMRI dataset to perform an event-related analysis (Fig. 6-right). The tool also provides video outputs representing the hemodynamic responses based on time variations of the events onset-time [11].

4. Impact

Considering the usage of UF^{2}C by the Neuroimaging community at the University of Campinas, we can conclude this software has had a significant impact on productivity and publications. As mentioned, since the first stable version (2014), 13 original research papers were published by 12 distinct authors with distinct backgrounds: biologists, physiotherapists, physicists, psychologists, and medical doctors. At the time of the submission of this paper, UF^{2}C has had more than 500 downloads, and based on original research publications, is already in regular use by two international centers, the University of Birmingham and the National Institute of Health of the United States.

5. Conclusions

In this article, we introduced and described the UF^{2}C-toolbox, which offers a user-friendly way to perform the most common types of fMRI BOLD signal studies, namely task-related and (resting-state) functional connectivity analyses. It also includes the EEG-informed fMRI analysis that is quite common in epilepsy investigations. The toolbox aims to facilitate the work of researchers who are beginning in this field and allowing experienced users to design and run large cohort analyses quickly. UF2C is free, open code, and is available for download, along with its manual at https://www.nitrc.org/docman/?group_id=1319.

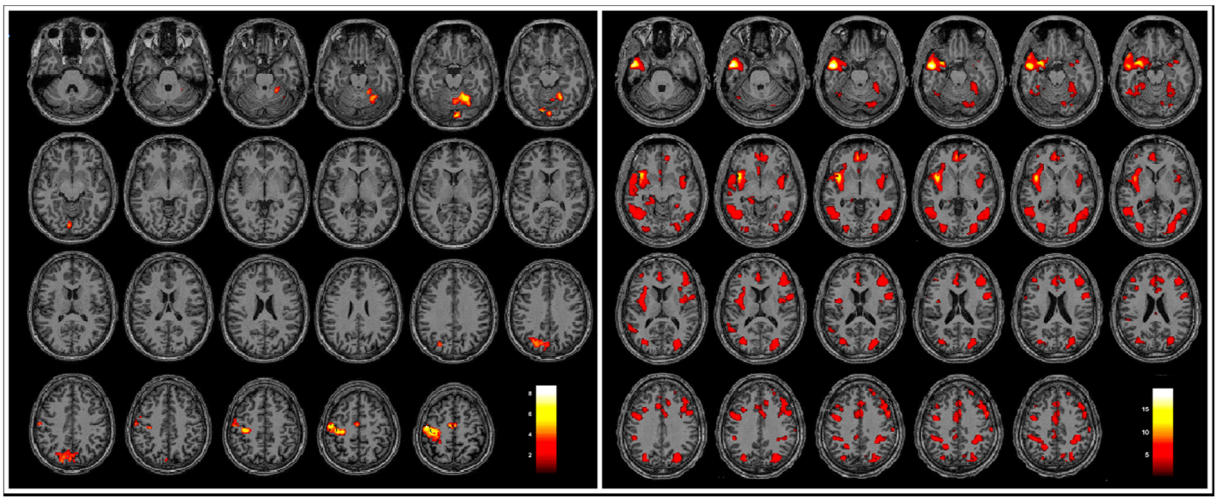

Fig. 6. On the left, a slice view of a hand-tapping task results preprocessed with the *fMRI Preprocessing* routine 3.2.1 and analyzed with the *First-Level Analysis (block design)* tool. On the right, a slice view result of an EEG-fMRI experiment preprocessed with the *fMRI Preprocessing* routine 3.2.1 and analyzed with the *EEG-fMRI Analysis* tool 3.2.2. The investigation on the right was performed in a patient with a left temporal pole focal cortical dysplasia, using the Brain Products MRI-Compatible EEG system. The EEG data was read in the BrainVision Analyzer Software, and the standard exported files were used as input in UF^{2}C.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This work was supported by FAPESP-São Paulo Research Foundation, Brazil [#2017-25795-7, #2013-07559-3]; Authors thank William M Wilson for English review.

Appendix A. Supplementary data

Supplementary material related to this article can be found online at https://doi.org/10.1016/j.softx.2020.100434.

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