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Short communication

MICA: A toolkit for multimodal image coupling analysis

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ABSTRACT

Background: Analytical methods of brain research involving across-voxel correlation between multimodal images are currently tedious and slow due to the amount of manual interaction required. We have developed a new software package to automate and simplify many of these tasks.

New method and results: Our software performs four primary functions to aid in research. First, it helps with consistent renaming of files preprocessed with other software, enabling more accurate analysis. Second, it automates ROI extraction using data from existing and custom brain atlases. Third, it performs coupling analysis to obtain across-voxel Pearson correlation coefficients between images of different modalities based on these brain atlases or custom ROIs. Fourth, it automatically performs multiple comparison correction to correct the P-value using two false discovery rate (FDR) methods and a Bonferroni method to reduce the false-positive rate.

Comparison with existing methods: Previous researchers have investigated the couplings between blood supply and brain functional topology in healthy brains and those from patients with type 2 diabetes, chronic migraine, and schizophrenia. These studies conducted analyses of both the whole and parts of the brain in terms of neuronal activity and blood perfusion, but the procedures were laborious and time-consuming.

Conclusion: We have developed a convenient and time-saving software package using MATLAB 2014a to automate the data preparation and analysis of across-voxel coupling between multimodal images.

1. Introduction

The noninvasiveness and high resolution of magnetic resonance imaging (MRI) means that MRI has become one of the most important methods to study brain function and disease. Previous studies have used multimodal imaging methods such as blood oxygen level dependent (BOLD) imaging (Biswal et al., 1995) and arterial spin labeling (ASL) imaging (Hendrikse et al., 2012) to explore brain changes caused by various diseases. However, researchers found that single-mode imaging techniques do not always comprehensively reflect specific functional states of the brain, as with the neurovascular coupling state involving both neuronal activity and cerebral blood perfusion (Attwell et al., 2010).

Liang et al. explored the relationship between functional network strength (FCS) and cerebral blood flow (CBF) in depth, and they found a tight coupling between the blood supply and brain functional topology, both during rest and in response to task demands (Liang et al., 2013). Zhu et al. found the CBF-FCS coupling in schizophrenia patients was lower than in healthy control subjects (Zhu et al., 2017). A previous study also investigated the relationship between positron emission tomography (PET) and MRI scans and showed that they reflected regional variations in the correspondence between glucose metabolism and measures of functional activity (Shokri-Kojori et al., 2019). In our previous studies, we found reduced neurovascular coupling in the limbic system and the default mode network in diabetic patients (Hu et al., 2019a), even though these patients did not experience cognitive impairment (Yu et al., 2019).We also found an abnormal neurovascular coupling state in the angular gyrus and the superior marginal gyrus in migraine patients (Hu et al., 2019b). These studies all investigated the across-voxel coupling between multimodal images and indicated the potential significance of this method for exploring the pathogenesis of brain disease.

Abbreviations: ALFF, Amplitude of low frequency fluctuation; ASL, arterial spin labeling; BOLD, blood oxygen level dependent; CBF, Cerebral blood flow; DTI, diffusion tensor imaging; FCS, Functional connectivity strength; ROI, Region of interest.

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However, the studies have all required a manual, labor-intensive procedure to obtain their results. A simple and useful analytical process is still needed to investigate relationships between images from different sources, especially for beginners. The ImCalc toolbox in the Statistical Parametric Mapping (SPM) 12 package (http://www.fil.ion.ucl.ac.uk/spm) can calculate the across-voxel correlation between two images, but it is very time-consuming and laborious to calculate the correlation coefficients one by one. For example, 100 subjects with 10 or more regions of interest (ROIs) requires at least 1000 mouse clicks and between 3 and 5 min for each one. A convenient and user-friendly automated software process is urgently needed to reduce potential mistakes introduced by manual selection.

Therefore, we present a pipeline using MATLAB 2014a (The MathWorks Inc., Natick, MA, US) to analyze the across-voxel coupling between multimodal images. Users can use either the ready-made Anatomical Automatic Labeling (AAL) atlas or the Harvard-Oxford atlas for analysis, or they can use custom ROIs from other atlases with the ROI extraction function in this software. Our software also provides multiple comparison correction methods to decrease the false positive rate.

2. Methods

The method for determining the coupling between multimodal images is to calculate the across-voxel Pearson correlation coefficients between the images. For example, for a given subject's brain, we process two images from different methods using a specific ROI mask and concatenate all voxel values from the extracted images into two large modality-specific vectors. We then correlate these two vectors to form a single value for this subject. Finally, we compare several groups of subjects statistically.

In our previous study, we calculated Pearson correlation coefficients of BOLD-based neural activity parametric maps and ASL-based CBF maps and conducted an inter-group comparison between healthy controls and patients with chronic migraines (Hu et al., 2019b). We found a general correlation between neural activity and blood flow perfusion at the whole brain level (Fig. A.1). In addition, we found this correlation state was abnormal in migraine patients compared to healthy controls by using a 2-sample *t*-test.

3. Implementation

We developed our MICA package in MATLAB 2014a and released it as an open-source package (https://github.com/hubolll/MICA_v1; or request to Dr. Bo Hu at rayhb@foxmail.com, or Prof. Wen Wang at wangwen@fmmu.edu.cn). The package also includes an example dataset and a user tutorial. The package uses SPM to read raw images, so users should install the SPM 12 toolbox (http://www.fil.ion.ucl.ac.uk/spm) in MATLAB before using MICA.

4. Data preparation

Our package does not provide preprocess data, so users need to preprocess the data in advance (e.g., by using DPABI) to correct head movements, standardize the data, and remove nuisance signals (Yan et al., 2016). The software only supports files in the common NIfTI format; other formats must be converted to this format before analysis. All of the images must have the same dimensions (e.g., $61 \times 73 \times 61$ voxels or $91 \times 109 \times 91$ voxels). In addition, irregular naming of the data may lead to an inconsistent file order, which may lead to incorrect matching (e.g., the image of subject A may be mistakenly matched with the image of subject B) during the calculations. Therefore, the software has a strict requirement for file naming: the names of the images for the same subject must match exactly. For example, to investigate the correlation between functional and structural images, the names of both images should be "Subject_001.nii".

5. Functions of the software

The interface of the software is shown in Fig. 1.

5.1. Removing the prefix from file name

Some data preprocessing software like DPABI automatically adds prefixes in an attempt to help the operator identify the data preprocessing steps. For example, the prefix "arwscf" means slice timing, realign, normalization, smooth, covariates regression, and band-pass filtering were successively applied on the raw data (Yan et al., 2016). To meet the file naming requirements of our MICA as described in the previous section, it is necessary to delete the prefixes of file names in batches. Our package leads users to select the data path and provide the number of characters to remove from the beginning of the file names. As shown in Fig. A.2, the ALFF images produced by DPABI require the removal of 10 characters to restore the original name of the data. Therefore, users put "10″ in the "number" field. Running the process copies the user's original data to a new folder with the prefixes removed.

5.2. Extracting ROIs from atlas

The software provides two sets of default ROIs: 116 brain regions based on the AAL atlas (AAL-116) and 112 brain regions based on the Harvard-Oxford atlas (HO-112). However, different brain atlases may reflect different aspects of brain function, so researchers may need brain regions using other atlases in their own research. The software performs automatic ROI extraction, segmenting the target brain atlas into single brain areas (Fig. A.3). For example, automated segmentation would use the AAL atlas to segment a single image into 116 files corresponding to 116 regions (Tzourio-Mazoyer et al., 2002). Alternatively, users can also use custom ROIs via a custom atlas.

5.3. Across-voxel coupling analysis

Coupling analysis is the core function of the software. Users select folders containing data in NIfTI format and then choose the brain atlas to use for analysis. As noted previously, we include two brain atlases, AAL-116 and HO-112. The provided atlases use an image size of $61 \times 73 \times 61$ voxels, so using the provided atlases requires all input images to be preprocessed to match. In addition, users can also add a series of custom ROIs (such as brain networks) themselves. The pre-defined ROIs restrict the analysis volume, with the brain images dot multiplied with the specific ROI to form the ROI-wise images. The package then calculates the across-voxel correlation coefficient between two ROI-wise images. Because the order of the results depends on the naming order of the custom ROIs, we suggest that the user number the custom ROIs in advance so that the results can be identified more efficiently. Finally, the user selects the output directory and output file name. The package writes the output results into a Microsoft Excel file containing an $M \times N$ matrix. Each row of the matrix represents a subject, while each column corresponds to an ROI, with the cell value representing the across-voxel correlation coefficient between the subject and ROI. Finally, for statistical comparison, the subjects of different groups should be analyzed separately.

5.4. Multiple comparison correction of results

It is common for multiple regions to be selected in step 2, which introduces the problem of multiple comparison. Our software provides three methods for multiple comparison correction: (i) the false discovery rate (FDR) method introduced by Storey (Storey, 2002); (ii) the FDR method introduced by Benjamin and Hochberg (the BH method) (Benjamini, 1995); and (iii) the Bonferroni method. Users input the result file names into the group 1 and group 2 boxes and then specify the statistical threshold (0.05 by default). After running the process, the corrected

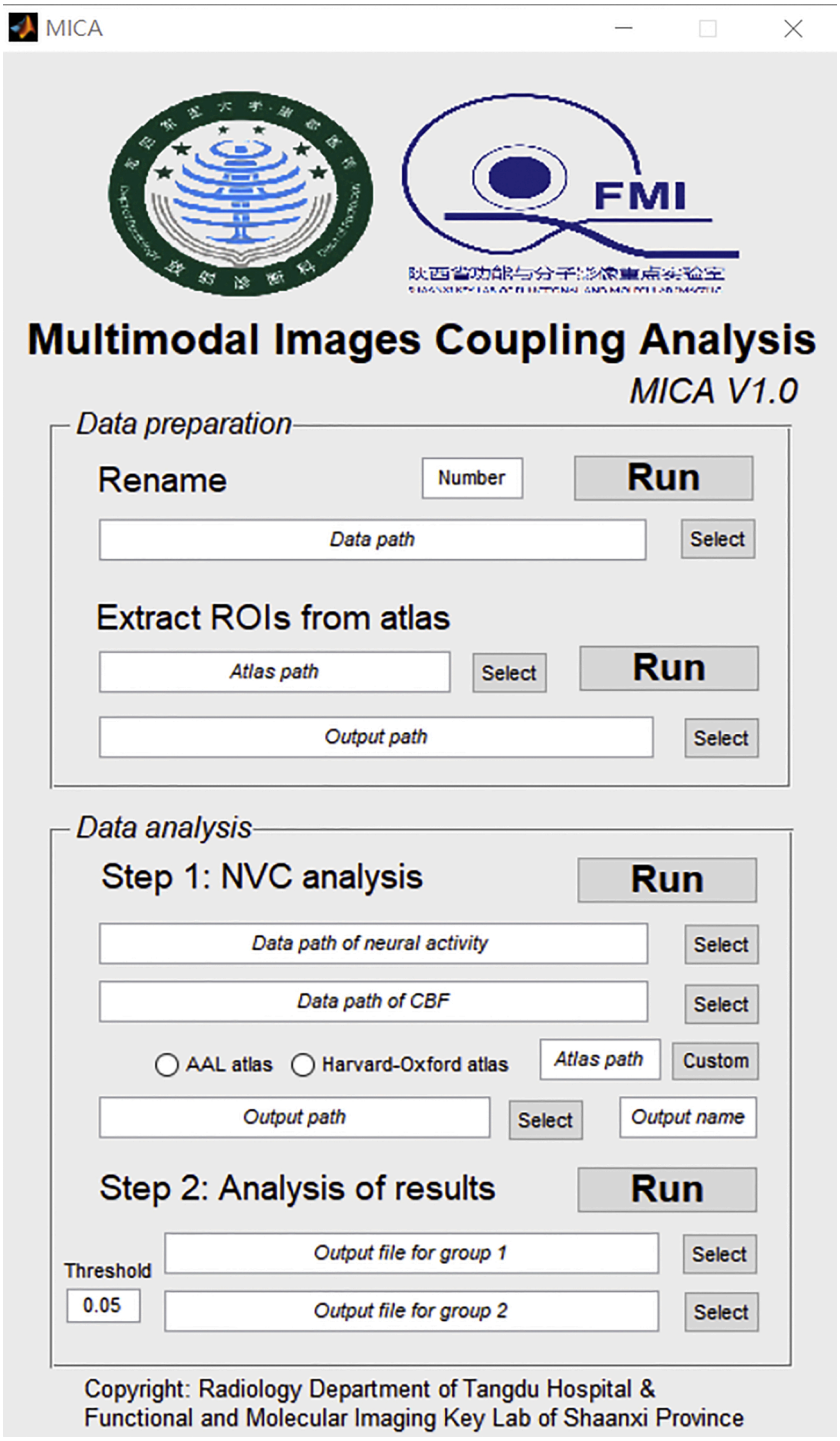

Fig. 1. The MICA user interface, which is divided into four parts to match the four broad tasks of the software (preprocessing, ROI extraction, neurovascular comparison, correction analysis).

results will be saved to the output folder. The result is a MATLAB file containing three variables: FDR1 representing the p-value corrected by the Storey method, FDR2 representing the value calculated by the BH method (Benjamini, 1995), and Bonferroni representing the value calculated according to the Bonferroni method.

6. Results

In a recent unpublished study, we compared the across-voxel neurovascular coupling before and after smartphone use (SPU). Twenty-one subjects recruited from the undergraduate cohort of our university underwent multimodal MRI scans (including BOLD, ASL, and T1WI scanning) on their first visit. After that, they were assigned to use a specific function module of their smartphone for 1.5 h. When this task was finished, the scans were repeated.

Amplitude of low frequency fluctuation (ALFF) (Zang et al., 2007) derived from the BOLD signal was used to represent neuronal activity, and cerebral blood flow (CBF) (Hendrikse et al., 2012) derived from the ASL signal was used to represent cerebral perfusion. Correlation coefficients between neuronal activity and perfusion maps were calculated

on each brain region in the automated AAL atlas (Liang et al., 2013). We used a paired *t*-test to compare them before and after SPU. We used the Bonferroni method for multiple comparison corrections ($p < 0.05$). After obtaining all of the results and performing multiple comparison correction, we used Sigmaplot (Systat Software, Inc, Point Richmond, CA) and BrainNet Viewer (Xia et al., 2013) to draw line graphs and brain graphs to illustrate the findings comprehensively (Fig. 2).

According to Fig. 2a, after the 1.5 -h SPU, ALFF-CBF couplings decreased in 4 of the ROIs that following the Bonferroni correction, including the left calcarine, the bilateral cuneus, and the right middle temporal gyrus (Nos. 43, 44, 45, and 86 in the AAL atlas). To visualize the results more directly, we projected the difference between the mean values before and after smartphone usage on the brain map (Fig. 2b). We then visualize the couplings between cerebral activity and blood perfusion affecting areas related to reading, visual information processing, and facial recognition.

7. Discussion

In this study, we have presented a new software page for analyzing the across-voxel correlation between brain images of different modalities. The software includes four main functions. First, it includes a data renaming function to remove the prefixes of file name generated by preprocessing software to facilitate data preparation. Second, it performs ROI extraction to divide brain images into individual ROIs using existing brain atlases or custom ROI maps. Third, it performs coupling analysis to produce across-voxel correlation coefficients between images of different modalities. Finally, it addresses the multiple comparison problem by correcting the *P*-value using two FDR methods and a Bonferroni method to reduce the false-positive rate. This software greatly simplifies the data processing for researchers studying the coupling relationship between multimodal images while providing great freedom in selecting ROIs.

Previous studies have explored the specific mechanisms of brain function and pathogenesis of brain diseases by combining images of different modalities, such as the identification of brain hubs (Liang et al., 2013) and the relationship between gray matter volume and metabolism (Hagmann et al., 2008). Two previous researches focused on the relationship between ASL imaging and resting state BOLD imaging to explore the degree of cerebral neurovascular coupling in type 2 diabetics (Hu et al., 2019a; Yu et al., 2019). Shokri-Kojori et al. studied the relationship between FCS using resting state BOLD images and cerebral metabolism using PET images and found the different rates of energy using among various brain networks as well as the changes in disease state (Shokri-Kojori et al., 2019). Many issues remain to be investigated, such as the relationship between long-term chronic hypoperfusion and brain structure remodeling, the relationship between task state brain function and brain structure remodeling (to explore the influence of daily cognitive training on brain structure), and the relationship between cerebral micro hemorrhage and cerebral functional hubs.

In addition, brain functional hyper-connectivity receives significant attention, exploring the correlation of BOLD signals in the same brain area from different subjects. Finn et al. explored the brain functional hyper-connectivity of subjects who were listening to an ambiguous story (Finn et al., 2018). They found an increased synchronization in the medial prefrontal lobe and amygdala in subjects with a high degree of paranoia. Consequently, researchers can also use this software to study across-voxel functional hyper-connectivity.

8. Conclusion

We have developed our convenient and time-saving software package to advance brain research. It is useful for data preparation and analysis of across-voxel coupling between multimodal brain images. It is our hope that this will enable greater use of automated data analysis in brain studies.

Ethics approval and consent to participate

The experiment conformed to the principles of the Declaration of Helsinki and was approved by the ethics committee of our hospital.

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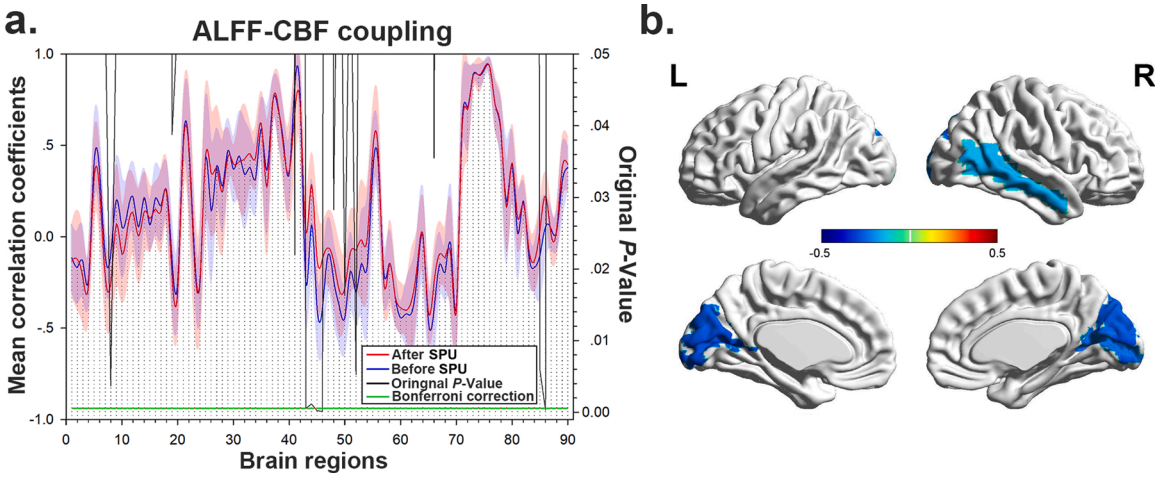

Fig. 2. The visualization of results. (a) Line graph of our unpublished smartphone use research. The blue and red lines indicate the mean correlation coefficients across all subjects. The shadows represent the standard deviations. The black line indicates the original P-value between two groups, and the green line is the threshold of statistical significance after Bonferroni correction. (b) Visual representation of the results. We pseudo-colored brain regions with significant inter-group differences using the intergroup difference value. Colors correlate with the signal in that brain region after subtracting the mean correlation coefficient between the two groups and projecting the difference between the mean value before and after smartphone use on the brain map.

CRediT authorship contribution statement

Bo Hu: Data curation, Formal analysis, Investigation, Methodology, Project administration, Software, Visualization, Writing - original draft. **Ying Yu:** Data curation, Funding acquisition, Methodology, Resources, Validation. **Wen Wang:** Conceptualization, Funding acquisition, Project administration, Software, Supervision, Writing - review & editing. **Guang-Bin Cui:** Conceptualization, Funding acquisition, Supervision.

Declaration of Competing Interest

The authors report no declarations of interest.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.jneumeth.2020.108962.

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