# Within- and between-subject reproducibility of matrix-based analysis of resting-state functional connectivity network

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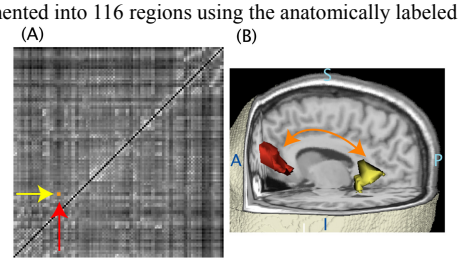
***Introduction***

Resting-state functional connectivity (RSFC) refers to the spontaneous temporal correlation, at a low frequency (< 0.1 Hz), of blood-oxygen-level-dependent (BOLD) signals between functionally related regions of the brain (Biswal et al., 1995). Many connectivity networks, including sensorimotor, visual, auditory, memory and default mode systems, have been identified by analyzing resting state fMRI data using analysis techniques such as the seed-voxel correlation and independent component analysis (ICA). Recently, a matrix-based analysis (MBA), based on 1) anatomic ROI segmentation (Tzourio-Mazoyer et al., 2002) followed by 2) pairwise correlations between anatomic regions, has been applied to the analysis of RSFC. The MBA is data-driven without a priori model, and is capable of quantifying the connectivity strength for the whole brain. Furthermore, it has been shown that the MBA can be applied to dissociate clinical populations (Liang et al., 2006; Liu et al., 2007). The MBA has a great potential to assist diagnosis and to follow-up treatment in clinical populations. However, the within- and between-subject reproducibility of MBA-based RSFC measurement has not yet been assessed, to our knowledge. The purpose of the study is to answer two questions: First, how reproducible are the functional connectivities measured by the MBA across multiple scan sessions for different participants? Second, which connectivity network demonstrates the highest reproducibility?

***Methods***

Six right-handed healthy volunteers (1F/5M, 21-49 years) were scanned on a 1.5 T MR scanner (GE Medical Systems) at rest. Each participant was scanned nine times, approximately eight weeks apart for more than a one-year period. A set of T1-weighted structural images covering the entire brain was acquired using a 3D-Spoiled Gradient Recalled (SPGR) sequence. For resting-state fMRI, EPI images covering the whole brain were acquired with the following parameters: axial orientation, TR/TE = 2500/50 ms, FA = 90°, acquisition matrix = 64 x 64 x 24, FOV = 24 cm x 24 cm, and slice thickness = 6 mm..

The data were processed using tools from the FMRIB Software Library (FSL, http://www.fmrib.ox.ac.uk/fsl) (Smith et al., 2004) and the Matlab codes developed in-house (MathWorks, Natick, MA). The functional images from each participant were aligned, low-pass filtered (< 0.1 Hz), detrended, and normalized to an MNI (Montreal Neurological Institute) template. For the analysis of functional connectivity, the fMRI data were segmented into 116 regions using the anatomically labeled template reported by Tzourio-Mazoyer et al. (2002). This parcellation divided the cerebra into 90 regions and the cerebella into 26 regions. Regional mean time series were estimated by averaging the time series of all voxels in that region. The Pearson's correlation coefficients were computed between each pair of brain regions, yielding 116 x116 correlation coefficients, for each participant in each session. These coefficients were stored in a 2D 116x116 matrix with only 6670 (116x115/2) unique elements (Figure A). Each element represents a functional connection between two brain regions (Figure B). For further statistical analysis, a Fisher's r-to-z transformation (i.e., $z = 0.5 \times \log[(1+r)/(1-r)]$) was applied to improve the normality of the correlation coefficients. The within-subject reproducibility across nine sessions was estimated by intraclass correlation coefficient (ICC), which is an ANOVA-based correlation measuring the relative homogeneity within groups in ratio to the total variation (Shrout and Fleiss, 1979). The between-subject reproducibility was assessed by coefficient of variance (CV), which is indicative of consistency of the data across subjects. The ICC and CV were estimated for each connection between brain regions, yielding 6670 ICC and 6670 CV values. An ICC $\geq$ 0.80 is considered outstanding reproducibility, 0.60 to 0.79 substantial, and 0.40 to 0.59 moderate (Landis & Koch, 1977). By convention, a CV $\leq$ 0.20 is considered acceptable for between-subject reproducibility.




***Results and Discussion***

Among the 6670 connectivity elements measured from every pair of the 116 anatomic brain regions, (1) 2896 elements (43.42%) have outstanding within-subject reproducibility across nine sessions, 2379 (35.67%) substantial, 737 (11.05%) moderate, and 658 (9.86%) poor; (2) 897 (13.45%) have acceptable between-subject reproducibility. The most reproducible connections between brain regions (i.e., ICC $\geq$ 0.80 and between-subject CV $\leq$ 0.20) are listed in the Table below, showing the correlation coefficients (CC), within-subject intraclass correlation coefficients (ICC), and between-subject coefficients of variance (CV). In summary, our results revealed reliable functional networks measured by the MBA across nine sessions over one year. Despite the fact that there exists between-subject variance, this technique proves to be useful for monitoring long-term changes in functional networks.

| Region 1 | Region 2 | CC | ICC | CV | Region 1 | Region 2 | CC | ICC | CV |
|---|---|---|---|---|---|---|---|---|---|
| SupraMarginal_R | SupraMarginal_L | 0.80 | 0.89 | 0.20 | Cingulum_Ant_R | Cingulum_Ant_L | 0.91 | 0.81 | 0.12 |
| Parietal_Sup_L | SupraMarginal_L | 0.69 | 0.80 | 0.19 | Cingulum_Mid_R | Cingulum_Ant_L | 0.78 | 0.85 | 0.19 |
| Parietal_Sup_L | Parietal_Inf_L | 0.85 | 0.89 | 0.18 | Cingulum_Mid_R | Frontal_Mid_R | 0.77 | 0.82 | 0.20 |
| Parietal_Inf_R | Parietal_Inf_L | 0.84 | 0.87 | 0.18 | Frontal_Mid_R | Frontal_Sup_R | 0.89 | 0.86 | 0.17 |
| Parietal_Inf_R | Temporal_Inf_R | 0.72 | 0.81 | 0.17 | Insula_L | Rolandic_Oper_L | 0.82 | 0.84 | 0.19 |
| Parietal_Inf_R | Parietal_Sup_R | 0.84 | 0.92 | 0.19 | Temporal_Sup_L | Insula_L | 0.78 | 0.81 | 0.17 |
| Temporal_Inf_R | Parietal_Sup_R | 0.80 | 0.83 | 0.19 | Temporal_Sup_L | Heschl_L | 0.84 | 0.81 | 0.18 |
| Cerebelum_Crus1_R | Parietal_Sup_R | 0.75 | 0.82 | 0.20 | Occipital_Mid_L | Occipital_Sup_R | 0.82 | 0.82 | 0.19 |
| Cerebelum_Crus1_R | Parietal_Inf_L | 0.67 | 0.81 | 0.17 | Occipital_Mid_L | Occipital_Inf_L | 0.85 | 0.84 | 0.20 |
| Cerebelum_Crus1_R | Paracentral_Lobule_R | 0.80 | 0.82 | 0.19 | Cerebelum_8_R | Cerebelum_8_L | 0.85 | 0.86 | 0.18 |
| Cerebelum_Crus1_R | Temporal_Inf_R | 0.81 | 0.83 | 0.19 | Frontal_Med_Orb_R | Frontal_Med_Orb_L | 0.90 | 0.85 | 0.17 |
| Cerebelum_Crus1_R | Cerebelum_Crus1_L | 0.92 | 0.90 | 0.18 | Rectus_R | Rectus_L | 0.87 | 0.82 | 0.19 |
| Cerebelum_6_L | Cerebelum_Crus1_L | 0.84 | 0.82 | 0.17 | Temporal_Mid_R | Temporal_Mid_L | 0.90 | 0.81 | 0.17 |
| Cerebelum_6_L | Cerebelum_4_5_L | 0.77 | 0.81 | 0.20 | Calcarine_R | Calcarine_L | 0.91 | 0.90 | 0.20 |
| Cerebelum_6_L | Cerebelum_6_R | 0.92 | 0.84 | 0.19 | Lingual_R | Lingual_L | 0.95 | 0.83 | 0.16 |
| Fusiform_L | Cerebelum_6_R | 0.81 | 0.82 | 0.18 | Caudate_R | Caudate_L | 0.83 | 0.86 | 0.16 |
| Fusiform_L | Fusiform_R | 0.89 | 0.91 | 0.20 | Pallidum_L | Putamen_L | 0.77 | 0.83 | 0.17 |
| Fusiform_L | Precuneus_R | 0.72 | 0.81 | 0.20 | Cingulum_Mid_L | Insula_R | 0.75 | 0.84 | 0.20 |
| Fusiform_L | Paracentral_Lobule_R | 0.68 | 0.82 | 0.20 | Postcentral_R | Rolandic_Oper_R | 0.79 | 0.81 | 0.18 |
| Precuneus_R | Precuneus_L | 0.96 | 0.91 | 0.16 | Frontal_Inf_Tri_R | Frontal_Inf_Oper_R | 0.86 | 0.91 | 0.18 |
| Paracentral_Lobule_R | Precentral_L | 0.80 | 0.83 | 0.18 | ParaHippocampal_R | Hippocampus_R | 0.78 | 0.80 | 0.18 |
| Parietal_Sup_L | Precentral_L | 0.74 | 0.84 | 0.19 | | | | | |

***References***

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