TEST-RETEST RELIABILITY OF FUNCTIONAL MOTOR CONNECTIVITY

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Introduction: Functional connectivity MRI (fcMRI) is used to measure inter-regional similarities in the time-varying BOLD signal across the resting brain and can yield important information about the connectivity of a network of functional anatomy, e.g., the motor system[1,2]. However, the reproducibility of these resting state networks both within and across sessions has yet to be clearly quantified, particularly with special consideration to physiological noise. In this study, we evaluate the consistency of resting state networks in five regions of the motor system: the primary motor cortices (LM1, RM1), the supplementary motor area (SMA), and the pre-motor areas (LPMA, RPMA), both within and across sessions for multiple participants using both voxel-wise and ROI-based approaches.

Methods: All imaging was performed on a Siemens 3T MRI scanner using an 8-channel receive-only head coil. Seven healthy participants (4 males, 3 females, mean age: 33±11 years) were imaged. T_2^*-weighted images were acquired using a single-shot EPI sequence (TE = 30 ms, TR = 2000 ms, FOV = 220 mm, resolution = 64 × 64) with 24 axial slices (sl. thick. = 6 mm) over 6 min 42 s that yielded 171 time points. A high resolution T1-weighted MPRAGE (TE = 3.44 ms, TR = 2250ms, TI = 900ms, flip angle = 9º, resolution = 256 × 256 × 96, FOV = 22 cm, sl. thick. = 1.5 mm) was also acquired for anatomic reference. Physiological data (cardiac and respiratory) was acquired during image acquisition using the output of an Invivo monitoring system (Invivo Corporation, Pleasanton, CA) which was digitized using a USB data acquisition module DT9801 (Data Translation, Marlboro, MA) connected to a Windows PC running customized software.

Each participant was imaged across three separate sessions. In each session, three resting state fcMRI acquisitions were acquired, with each being separated by two block-design fMRI acquisitions (motor and visual activation paradigms - 8 On-Off blocks of 20 seconds-On and 20 seconds-Off). During the motor activation experiment, the participant performed a self-paced finger-thumb apposition task.

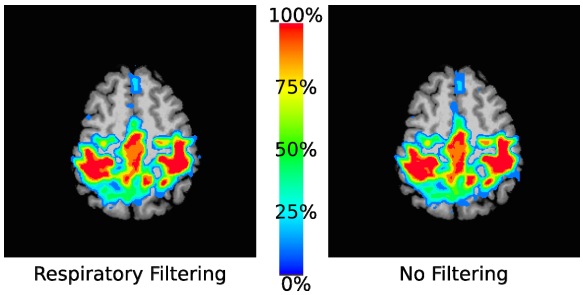

Figure 1. % overlap of fcMRI acquisitions for a representative subject over 9 resting state scans during 3 separate sessions.

Data were analyzed using AFNI (Robert Cox, NIH) and MATLAB (MathWorks Inc., Natick, MA). Each subject's functional images were corrected for slice timing, optionally filtered to remove physiolocal artifacts[3], and registered to the first functional scan from their first session. A 6mm FWHM Gaussian blur was applied to the registered functional scans. To chose seed voxels for the fcMRI analysis, each subject's functional motor scan from their first session was deconvolved with the motor task function. The motor activation *t*-statistic image was then thresholded so that the single most active cluster in the LM1 contained 50 voxels. The time series obtained from these seed voxels in the LM1 were averaged and used for deconvolution with the resting state data of the whole brain to obtain fcMRI images (R^2 images thesholded at 0.4). This process was repeated for the physiology filtered resting state data. Spherical ROIs were manually drawn in the LM1, RM1, RPMA, LPMA, and SMA and masked with the fcMRI image generated from the concatenation of the resting data from the first session. These composite regions were used to extract average time series from each ROI within the resting-state scans for further statistical analysis.

The statistical methodology was based upon large sample theory of stationary signals. Given that the signal lengths are relatively large, we estimated the exact asymptotic variance matrix for the cross-correlations of the stationary signals. We used the asymptotic variance to normalize the cross-correlations to obtain approximately independent normal variates. Within each subject the signals were taken to be potentially dependent, whereas across subjects they are independent. We looked at the pairwise connections and tested for all contrasts with respect to subject, session and scan effects. We performed a two-way MANOVA using PROC GLM in SAS (SAS Institute, Inc., Cary, NC) to identify session and scan effects. Due to the exact normalization of the signals the observations for individual connections become approximately independent allowing us to choose a threshold of 0.0051 after adjusting for multiple comparisons.

	LM1	LPMA	RM1	RPMA	SMA
LM1	X	0.6374 / 0.8115	0.9715 / 0.9795	0.6698 / 0.5828	0.3904 / 0.3380
LPMA	X	X	0.1080 / 0.2360	0.8870 / 0.7982	0.4784 / 0.5531
RM1	X	X	X	0.4699 / 0.3617	0.0487 / 0.0402
RPMA	X	X	X	X	0.6437 / 0.5432

	LM1	LPMA	RM1	RPMA	SMA
LM1	X	0.7751 / 0.5769	0.0884 / 0.0935	0.9292 / 0.5614	0.1106 / 0.1438
LPMA	X	X	0.0222 / 0.0454	0.2802 / 0.2677	0.2448 / 0.2142
RM1	X	X	X	0.2646 / 0.8528	0.0187 / 0.0329
RPMA	X	X	X	X	0.8901 / 0.8132

	LM1	LPMA	RM1	RPMA	SMA
LM1	X	0.4919 / 0.6541	0.6139 / 0.6689	0.7431 / 0.7031	0.8206 / 0.7520
LPMA	X	X	0.7701 / 0.8942	0.6109 / 0.5645	0.0834 / 0.1418
RM1	X	X	X	0.5930 / 0.5933	0.4983 / 0.4187
RPMA	X	X	X	X	0.0636 / 0.0877

	LM1	LPMA	RM1	RPMA	SMA
LM1	X	0.0020 / 0.0021	0.0184 / 0.0314	0.0362 / 0.0118	0.0004 / 0.0001
LPMA	X	X	0.0001 / 0.0001	0.2466 / 0.1760	0.0005 / 0.0001
RM1	X	X	X	0.0001 / 0.0001	0.0001 / 0.0001
RPMA	X	X	X	X	0.0001 / 0.0001

Table 1. *p*-value tables of two-way MANOVA for inter-regional connectivity. **Upper-left**, Session effect, **Upper-right**, Scan effect, **Lower-left**, Session*scan effect, **Lower-right**, Subject effect. High p-values (>0.005) indicate highly reliable functional connectivity.

Results and Conclusion: Figure 1 shows a voxel-wise measure of overlap in the motor system network of one representative subject with and without physiological filtering. This image shows the percent overlap of each fcMRI acquisition across all sessions (9 scans total). A high degree of overlap is present showing reliable identification of the motor connectivity in M1 and SMA, although no significant visual difference is present as a result of physiological filtering. The output of the two-way MANOVA analyses are shown in Table 1. These tables show *p*-values which indicate a significant influence of the session, scan, and subject on the interregional connectivities of the resting motor network. Overall these results indicate that there is a high degree of connectivity between all the regions within a subject and that it is not influenced by the visit (session) or the scan number within a visit, and this holds true whether the physiological signal is filtered or not. Filtering the physiological data from the original data did not significantly change the reliability. The results also indicate that while the resting state networks are quite reliable within a single subject, there is a significant variation across subjects. This implies that although the connections between the various systems within a subject are quite reliable, the strength of these connections are not the same across subjects. These results have a significant impact on the design of both large scale and longitudinal studies that rely on changes in the resting state signal following rehabilitation or therapeutic intervention.

References: **1.** Biswal et al. MRM, 1995;34:537-541. **2.** Xiong et al. Hum Brain Mapp, 1998;8:151-156. **3.** Glover et al. MRM, 2000;1:162-167.