FUNCTIONAL CONNECTIVITY OF THE MOTOR CORTEX: TEST-RETEST RELIABILITY

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Introduction: Functional connectivity of the resting brain has become a subject of numerous studies. It can be used to evaluate the strength of neuronal connections in healthy individuals and patients with various disorders. Rombouts *et al.* showed that the activity in the default mode network is altered in patients with mild cognitive impairment, and may act as an early marker to detect Alzheimer's disease[1]. Waites *et al.* observed that although activation patterns may be similar between normal subjects and patients with left temporal lobe epilepsy, the resting state functional connectivity was significantly different[2]. The reproducibility of the resting state networks within and across sessions has yet to be quantified. In this study, we evaluate the spatial consistency of resting state networks, in the primary motor cortex, both within and across sessions for each subject using seed voxel analysis.

Methods: Seven healthy subjects (4 males, 3 females, mean age: 33±11 years) participated in this study. Functional images were acquired using single-shot EPI T_2^*-sensitive sequence (TE = 30 ms, TR = 2s, 3.4 × 3.4 mm^2 in-plane resolution and a FOV of 22 cm) using 24 axial slices (6 mm thick) with no gaps between slices. A high resolution T_1-weighted MPRAGE (TE = 3.44ms, TR = 2250ms, TI = 900ms, flip angle = 9°, 96 slices, slice thickness 1.5 mm, 0.86 × 0.86 mm^2 in-plane resolution and a FOV of 22 cm) was acquired for anatomic reference. All images were obtained on a Siemens 3T Tim Trio scanner using the 8-channel head coil.

Each subject underwent three rest scans in three separate sessions for a total of nine resting state scans. In each session the three resting state scans were separated by two functional activation scans involving the motor cortex and visual cortex respectively. During the resting state scans, the subjects were instructed to close their eyes and relax while refraining from falling asleep. The first functional scan was a self paced finger-thumb apposition task and the second functional scan was a visual task where the subject viewed a flashing checkerboard at a frequency of 5 Hz followed by a blank screen. Block design was used for both scans with 20s-On and 20s-Off for a total of 8 cycles each. The cardiac and respiratory data were collected using the output from an Invivo monitoring system (Invivo Corporation, Pleasanton, CA) which was digitized using a USB bases data acquisition module DT9801 (Data Translation, Marlboro, MA).

Data were analyzed using AFNI (Robert Cox, NIH) and MATLAB (MathWorks Inc., Natick, MA). Images were first registered, intensity normalized and spatially normalized to the Talairach coordinate system. For fMRI analysis, the general linear model (GLM) was used to determine the voxels that were significantly correlated with the task using a boxcar function convolved with the hemodynamic response function. For fcMRI, seven different sets of seed voxels (5, 10, 15, 20, 50, 100 and 200 voxels) which exhibited the highest *t*-scores from the fMRI activation maps were selected from the motor cortex. The resting state time series from these voxels were averaged and the resulting time series was used as a regressor in the GLM analysis of resting state data. The functional connectivity maps thus obtained were thresholded at $P<0.05$ and only voxels within the primary motor cortex were considered for further analysis. Reproducibility index, *Roverlap*[3] was calculated between the three scans for each session. *Roverlap* reflects the proportion of highly correlated voxels overlapping across the scans and is given as

$$Roverlap = \frac{1}{3}\left[\left(2\times\frac{Vscan1\cap Vscan2}{Vscan1+Vscan2}\right)+\left(2\times\frac{Vscan2\cap Vscan3}{Vscan2+Vscan3}\right)+\left(2\times\frac{Vscan1\cap Vscan3}{Vscan1+Vscan3}\right)\right]$$

, where $Vscan1$, $Vscan2$ and $Vscan3$ represent the number of highly correlated voxels ($P<0.05$). To measure the consistency across sessions, the coefficient of variation (COV=standard deviation/mean) of the reproducibility index was calculated for each subject across three sessions. To assess the confounding effects of the physiological noise on the reliability of connectivity maps, the resting state data were filtered using RETROICOR with the prospectively acquired physiological data as its input[4].

Results: The functional connectivity maps obtained before and after filtering in the primary motor cortex of the resting brain were fairly consistent as shown for one of the subjects after physiological filtering in Figure 1a. Figure 1b shows the mean *Roverlap* (average of *Roverlap* across three sessions) increasing as the number of seed voxels is increased for the same subject and reaches its peak value of 0.66 and 0.65 for the original and filtered data respectively. Removal of physiological noise reduces the mean *Roverlap* comparatively; however the standard deviation of the mean *Roverlap* decreases after filtering. Similarly, the mean coefficient of variation (average of COV for seven subjects) of *Roverlap* decreases as the number of seed voxels is increased and reaches its minimum value of approximately 9.5% when 100 seed voxels are selected as shown in Figure 2. A further decrease to approximately 8.7% is seen when 200 seed voxels are selected for the filtered data. Filtering does not significantly change the mean coefficient of variation of *Roverlap*, however the overall standard deviation due to the physiological noise is reduced after filtering.

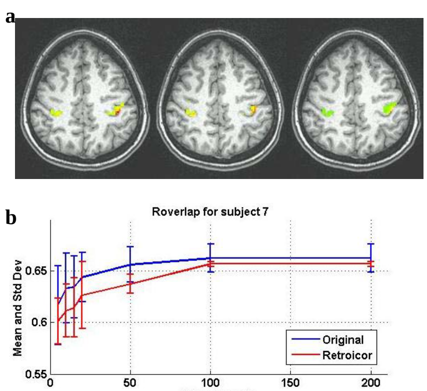

Figure 1: (a) Functional connectivity maps for subject seven, rest scan 1 after physiological noise filtering using 100 seed voxels **(b)** Mean *Roverlap* within session for subject seven before and after physiological noise filtering for seven different sets of seed voxels.

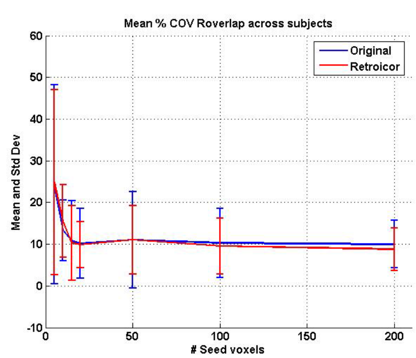

Figure 2: Mean coefficient of variation across sessions; for seven different sets of seed voxels before and after physiological noise filtering

Conclusion: The functional connectivity maps are consistent within and across sessions and there is a decrease in the number of overlapping voxels after filtering out the physiological noise. In addition, the selection of appropriate number of seed voxels is essential in studies involving functional connectivity. Our results suggest that at least for the motor cortex, choosing about 100 seed voxels improves the reliability of the functional connectivity maps. Although not significant, physiological filtering removes some of the variability in the results.

References: **1.** Rombouts et al. Hum Brain Mapp, 2005; 26(4):231-240. **2.** Waites et al. AJNR, 2006; 59:335–343 **3.** Miki et al. AJNR, 2000; 21:910-915 **4.** Glover et al. MRM, 99-4512