

# The functional anatomy of SMA at rest: Clustering and Connectivity independently measured with DTI and RS-fMRI

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## Introduction

In the last decade, there is increasing evidence that low frequency components of the BOLD response of functionally associated areas are correlated. This was first shown by Biswal et al. [1], who used functionally defined areas in the motor system for a classical correlation analysis. Another approach was developed by Salvador et al. [2], who used an anatomically defined template to define seed regions for a partial correlation analysis. Recently, more data-driven methods, such as ICA [3], have been used to distinguish the networks active in the brain. They have thus far only shown large networks of brain areas, which leaves the question open, at what level the brain is organized in resting state. In other words, if we would be able to subdivide regions of these networks meaningfully on the basis of anatomical or functional grounds, would each subregion be distinguished by its own unique activity pattern in resting state?

To answer this question, we have focused on the supplementary motor area (SMA). In a previous study [4] it was shown that SMA is subdividable into two functionally different regions, namely SMA-proper and preSMA, on the basis of tractography patterns obtained in a Diffusion Tensor Imaging (DTI) analysis. In this study, we have used this established parcellation scheme to investigate whether the thus defined subregions have distinct resting state time courses. Moreover, we have developed new methods to parcellate SMA on the basis of the resting state data alone and we have studied the resting state connectivity patterns from the resulting clusters.

## Methods

**Data acquisition:** After informed consent was given, eight subjects participated in a combined DTI and resting state study. The subjects were scanned on a 3 T Siemens Trio scanner, using the eight-channel phased array head coil. Diffusion weighted data were acquired by using a twice-refocused spin-echo EPI sequence [5] with the following imaging parameters: TR 8000 ms, TE 88 ms, 70 slices, matrix size 96x96, resolution 2.0x2.0x2.0 mm, 64 directions, b-value 1000 s/mm<sup>2</sup>, 1 volume without diffusion weighting, bandwidth 1930 Hz/pixel, scan time 9 min. Resting-state data were acquired by using gradient-echo EPI with the following imaging parameters: TR 1400 ms, flip angle 67° to conform to the Ernst angle for this TR, TE 30 ms, 21 slices, matrix size 64x64, resolution 3.5x3.5x5.0 mm, distance factor 20%, 1030 volumes, bandwidth 1815 Hz/pixel, scan time 25 min.

**Image analysis:** Diffusion data were analyzed in FSL. These data were first corrected for eddy currents and head motion. Then probability distributions on fiber directions were calculated at each voxel, using the new two-fibre model, published by Behrens [6]. The SMA-ROI was defined on the basis of coordinates in standard space, following procedures in [4]. In our analyses, we have used three sagittal slices (at 0, 2 and 4 mm from the midline), surrounding the original slice in [4] at x = 2 mm. This was done to increase the sample size for the clustering analysis in the resting state part. The clustering was done as in [4], using the k-means algorithm. The first six volumes of the resting state scans were discarded to compensate for T1 relaxation effects. The remaining volumes were aligned and then normalized to the EPI template in standard space. In the first analysis, mean time courses were calculated from the DTI subdivisions. The correlation of each these time courses with all the voxels in the complete ROI was then calculated to establish whether there was a clear subdivision between the two areas. To investigate if the correlation structure changes over different frequency bands, we have used the discrete wavelet transform (DWT), which creates a time-frequency representation of the signal. We have computed frequency-band-specific correlations for each voxel to each ROI time course. The DWT combines the creation of a time-frequency representation of the signal with a subsampling operation at each band to reduce the data amount without loss of information. In the resting-state clustering analysis, we have used these subsampled band-pass filtered signals to cluster the resting state data using the fuzzy c-means algorithm. We have used the mean time courses of the resulting clusters as regressors in a fixed effects analysis over all subjects, to establish consistent resting state connectivity maps with these clusters.

## Results

We were able to successfully cluster SMA into two regions on the basis of the DTI data in all 8 subjects. In figure 1a the result of a typical subject is shown. From the analysis of the correlations in different wavelet scales between the ROI time courses and the ROI-voxels, we have concluded that scale 4 is a suitable scale to perform clustering in, because in this scale, the voxels show the clearest separation between SMA-proper and preSMA. The results of the fuzzy c-means clustering in this scale are shown in figure 1b, in which the color scale is membership, ranging between 0 and 1. Two clearly defined clusters can be seen, whose borders to a large extent coincide with the DTI-defined borders. Over all subjects the overlap between the DTI and the resting state clusters was  $80 \pm 8\%$ . The results of the resting-state connectivity analyses on the basis of these clusters are shown in figure 1c ( $p = 0.05$  FWE-corrected). SMA-proper and preSMA show clearly distinguishable correlation patterns: correlations with SMA are mainly restricted to motor areas, while areas within the prefrontal and parietal areas (among others) correlate with the preSMA cluster. The correlation pattern with the whole area shows that to a large extent the SMA-proper/preSMA connectivity is a subset of the connectivity with the whole area. This can be explained by the fact that in most subjects, the SMA-proper and preSMA are almost completely orthogonal. Thus we have shown that the subdivision between SMA-proper and preSMA also exists in resting state fMRI and that it can be established by clustering resting-state data alone.

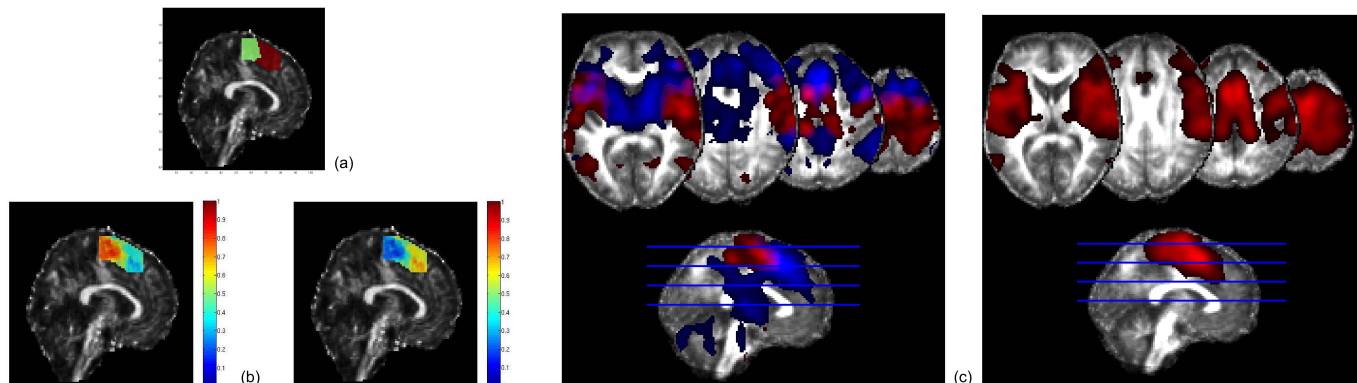


Figure 1a. Results from k-means clustering on the DTI data of one subject. 1b. Results of the fuzzy c-means clustering in the same subject (left membership of SMA, right of pre-SMA). 1c. Resting state connectivity maps of SMA (red) and preSMA (blue) shown on left and of the whole area shown on right.

References: 1. Biswal, B. et al. *MRM* 1995. **37**(4): p.537-41. 2. Salvador, R. et al. *Cer Cor* 2003. **15**(9): p. 1332-42. 3. Beckmann, C.F. et al. *Phil Trans R Soc B* 2005. **360**: p. 1001-13. 4. Johansen-Berg, H. et al. *PNAS* 2004. **101**(36): p. 13335-40. 5. Reese, T.G. et al. *MRM* 2003. **49**(1): p. 177-82. 6. Behrens, T.E. et al. *NI* 2006. **34**(1): p. 144-55.