**Functional connectivity dynamics differentiate subdivisions of precuneus**

1. **Hypothesis**

The whole brain functional connectivity of each subdivision of the precuneus will change over time, alternating between a finite number of “micro-states”. We propose that each subdivisions will posses a distinct microstate profile (e.g., number of states, state features, state-shifting pattern, duration of states).

**B. Analysis procedures**

1. Preprocessing

Chao-gan has preprocessed the first 5 min of session 1 and session 2 data for all 25 subjects.

The data I am going to use are: HeadMotionRegression\_2Friston\_24\_COV\_GloabalWS (motion corrected with Friston 24, global signal regressed out. W: normalized, S: smoothed).

2. ROI Time series extraction

a. Seed locations were determined based on Margulius et al. (2009)

Seed 4 (-2/-36/35): Located in the middle of the splenium in the *y*-axis, within the cingulate gyrus.

Seed 6 (-2/-47/58): Located at the center of the marginal ramus of the cingulate sulcus, 6 mm posterior to it.

Seed 14 (-2/-64/45): Located ventral to seed 13 along the sulcus.

Seed 17 (-1/-78/43): Located ~10 mm posterior to seed 13 and 6 mm anterior to the parietal-occipital sulcus.

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b. ROI selection:

connectivity atlas: Crad-200

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structrual atlas: Harvard-Oxford atlas 110 cortical regions

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Why choose this atlas

c. Time series extraction:

The four seeds were located within four ROIs in Crad-200. The time series of all seeds and 196 ROIs (the four ROIs overlapped with the seeds were removed) were extracted from the preprocessed 4D timeseries data in MNI space.

3. Stationary FC analysis using the full length of the time series

For each seed, preform the following analysis. Repeat a-e for seed 2-4.

1. Calculate the inversed covariance matrix using matlab code
2. Average the inversed covariance matrix across all subjects
3. Inverse Fisher transform the inversed covariance matrix.
4. Correct the results for multiple comparisons using false discovery rate (q < 0.05).
5. Sort connections based upon lobar (i.e., frontal, temporal, parietal, occipital, subcortical) classifications and create a 1 x N correlation matrix. N is the number of ROIs.

4. Dynamic FC estimation using time series within a sliding window:

Sliding window creation

1. The window can be created by convolving a rectangle (width = 0.645 s \*22 TRs =45s) with a Gaussian (and slide in steps of 1 TR, resulting in W= N-width+1 windows (Allen, et al., 2012).
2. rectangular window
3. Gaussian window
4. Hanning
5. Hamming window

FC analysis for each sliding window

For each seed perform the following analysis and repeat a-c for seeds 2-4

1. repeat the analyses in step 3 for each sliding window
2. plot the correlations between the seed and the significant ROIs as a function of time (each time point corresponding to the center of the window)
3. plot the power spectral for the time series in c.

FC state identification using clustering analysis

FC state can be defined as highly structured patterns of FC that emerge and dissolve over tens of seconds (Allen et al., 2012).

1. choose exemplars defined as those windows with local maxima in FC for each subject
2. apply K-means clustering algorithm to the set of exemplars from all subjects to obtain the group centroids. The number of clusters was determined using the elbow criterion of the cluster validity index, computed as the ratio between within-cluster distance to between-cluster distance. The centroids of a cluster putatively reflects a connectivity state stably present within the data.
3. Repeat this clustering for 500 times to increase the chances of escaping local minima.
4. Estimate the network modularity of each state by using the Louvain algorithm implemented in the Brain Connectivity Toolbox.
5. Repeat the Louvain algorithm on 100 bootstrap resamples (resampling subject exemplars within each cluster) to obtain the occurrence trend and the best linear fit line.
6. Plot the state transition examples for several subjects and compute the average transition matrix (TM). Approximate the stationary probability vector () as the principal eigenvector of the TM, which represents the expected behavior of the system in the long run.

6. Compare the number, feature, and duration of states among the 4 seeds

7. Calculate the test-retest reliability of the state analysis. This can be quantified as intra-class correlations on state measures between the data collected in session 1 and session 2.