Dynamic Resting-state Functional Connectivity in Awake Animals

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Introduction: It has been increasingly recognized that resting-state functional connectivity (RSFC) is dynamic in nature. The research of dynamic RSFC in humans has yielded promising results (1, 2). However, very little about dynamic RSFC in animals is known. In the present study we utilized the single-volume co-activation method (2) to identify nonstationary patterns of spontaneous blood-oxygenation-level dependent (BOLD) fluctuations in the awake rat brain. In addition, we examined the temporal evolution of the co-activation patterns.

Method: Resting-state fMRI data of 42 male Long-Evans rats were acquired in previous studies (3-7), and re-analyzed for the purpose of this study. Briefly, rats were imaged at a 4.7 T Bruker scanner at the awake condition (gradient echo EPI, TR=1s, TE=30ms, flip angle=60º, matrix size=64*64, FOV=3.2*3.2cm, 18 1mm thick slices). Functional images were preprocessed with conventional procedures including registration to a segmented rat brain atlas, motion correction, spatial smoothing, regression of motion parameters and white matter/ventricle signals, as well as band-pass filtering (0.002-0.1 Hz). The BOLD signal was normalized to its standard deviation for each voxel. The anatomically defined bilateral infralimbic cortex (IL) and the unilateral primary somatosensory cortex barrel field (S1BF) were selected as seeds based on Swanson rat atlas. For each seed, the regionally averaged time series was extracted, and 15% frames with the highest signal intensity in each time series were averaged to generate mean maps. In addition, these frames were clustered into 3 co-activation patterns (CAPs) based on their spatial similarity by using k-means clustering (Fig. 2). To evaluate the temporal evolution properties of these CAPs, the averaged map of each CAP was used as a template to calculate the spatial correlation with individual frames. The correlation coefficient with each CAP template for each frame was thresholded at 0.2 ($p<10^{-6}$). The time 0 (t=0) was defined at correlation coefficient peaks, and 5 frames before and 6 frames after t=0 were selected and these 12 frames together were considered as one epoch for a particular CAP. All epochs were then averaged for each CAP. Standard errors of individual time points across epochs were also calculated to evaluate the consistency in temporal patterns for each CAP.

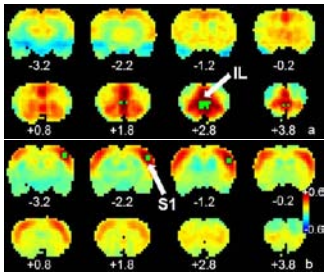

Fig.1: Averages of 15% frames with the highest BOLD signal intensity. Seeds were marked by green color and white arrows. a, IL seed map. b, S1BF seed map. Color bar represents BOLD signals normalized to standard deviation. Distance to Bregma is marked below each slice.

Results: The IL map showed robust co-activation regions like anterior cingulate, caudate putamen, motor and somatosensory cortices (Fig.1 a). The S1BF map revealed robust bilateral co-activation in somatosensory and motor cortices (Fig. 1b). These co-activation patterns well agreed with the connectivity maps revealed by the correlational analysis (4), confirming that the overall connectivity pattern can be readily revealed by only 15% of rsfMRI data (2). The 15% frames selected were further clustered into distinct CAPs for each seed (Fig. 2). For IL, the strongest co-activation occurred in cingulate and caudate putamen for CAP1 (Fig. 2a) and in cortical regions for CAP2 (Fig. 2b). CAP3 showed a pronounced long-distance co-activation in hippocampus (Fig. 2c). Similarly, for S1BF, distinct CAP patterns were revealed with CAP1 in prefrontal region and caudate putamen (Fig. 2d), CAP2 in bilateral motor and somatosensory cortices (Fig. 2e) and CAP3 in hippocampus (Fig. 2f). Other cluster numbers were also tried and results were generally consistent (data not shown). For three IL CAPs, Fig. 3 showed the temporal evolutions of spatial patterns, and these patterns were consistent across epochs as indicated by small error bars at each time point (Fig. 4).

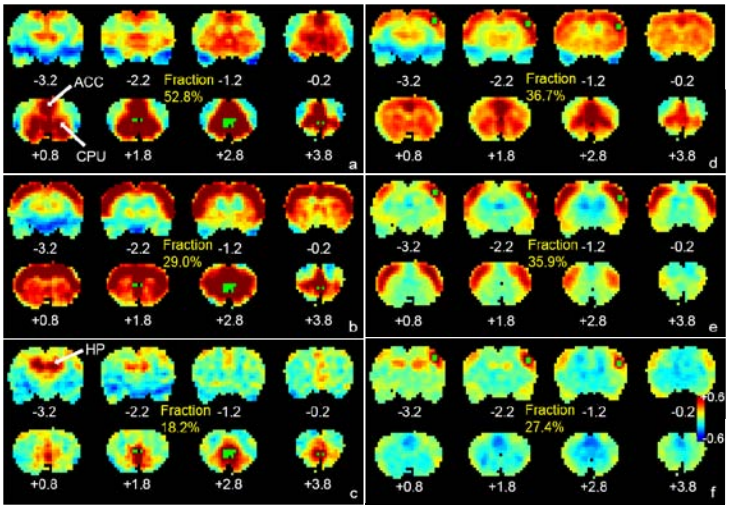

Fig. 2. Distinct spatial CAPs in top 15% frames. Left (a-c), infralimbic seed. Right (d-f), S1 seed. The occurring rate of each CAP is labeled. ACC, anterior cingulate. CPU, caudate putamen. HP, hippocampus.

Conclusion and discussion: The results validated the single-volume co-activation method in awake rat RSFC data. Averaged co-activation patterns from 15% frames with the highest signal intensity closely resemble seed-based functional connectivity in IL and S1BF obtained by correlational analysis (4), and k-means clustering further revealed distinct CAPs that are not apparent in the averaged maps. Importantly, those CAPs also have consistent spatiotemporal patterns, which was not explored before. In conclusion, the current study extended the single-volume co-activation method and revealed both spatial and temporal patterns of specific functional neural circuitries in the awake rat brain.

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References: 1. Hutchison et al., NeuroImage 80: 360-78. 2. Liu and Duyn, PNAS 110(11):4392-7. 3. Liang et al., NeuroImage 83:237-44. 4. Liang et al., Neuroimage 59(2):1190-9. 5. Zhang et al., J Neuroscience Met: 189(2): 186–196. 6. Liang et al., J Neuroscience: 31(10):3776 –3783. 7. Liang et al. J Neuroscience: 32(30): 10183-10191.

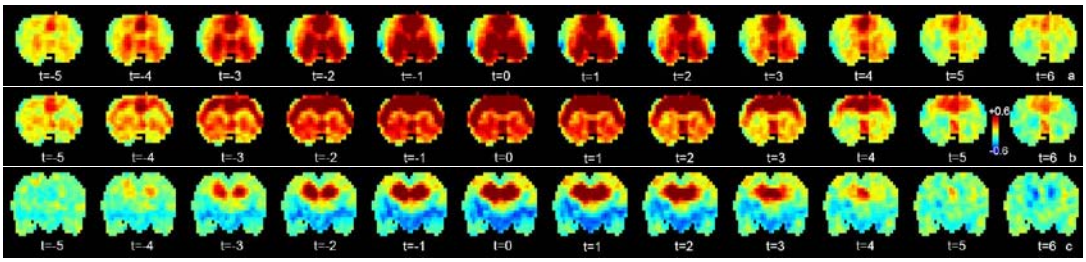

Fig. 3. Averaged temporal evolution for three IL CAPs. a-c, CAP 1-3. t=0 (in second) is the peak of correlation with each CAP template. Distance to Bregma: a and b +0.8mm, c -3.2mm.

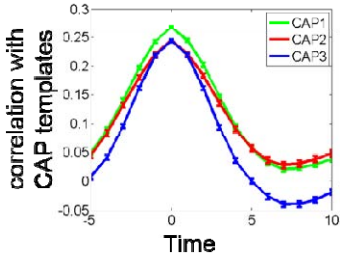

Fig. 4. Consistent temporal dynamics for 3 IL CAPs. t=0 (in second) is peak of correlation with each CAP template. Error bar shown in standard error of means (SEM)