Anticorrelated Resting-state Functional Connectivity in Awake Rat Brain

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Introduction Resting-state fMRI (rsfMRI) measures spatial patterns of functional connectivity across the brain by detecting temporal correlations of low-frequency spontaneous fluctuations of the blood-oxygenation-level dependent (BOLD) signal. Conceptually, temporal correlations of spontaneous BOLD fluctuations between functionally connected brain regions should include both positive and negative values. The vast majority of RSFC studies have been focused on positive RSFC, whereas our understanding about its conceptual counterpart—negative RSFC (i.e. anticorrelation)—remains elusive. To date, anticorrelated RSFC has yet been observed without the commonly used preprocessing step of global signal correction. However, this step can induce *artifactual* anticorrelation[1,2]. Therefore, validating the existence of anticorrelation that is independent of preprocessing methods is of critical importance, particularly negative RSFC might represent a group of functional connections with a distinct neurophysiologic mechanism and thus could be crucial for better understanding of neural circuitries and brain diseases. In this report we demonstrated robust anticorrelated RSFC in a well characterized frontolimbic circuit between the infralimbic cortex (IL) and amygdala in the awake rat.

Method Twenty four adult male Long-Evans (LE) rats (300 – 400 g) were used in this study. Rats were acclimated to MRI restraint and noise as previously described[3]. All animals were imaged at the awake condition for RSFC analysis using the awake animal imaging model we established [4-6]. 16 (out of the 24) rats underwent the imaging session in the anesthetized condition at minimal 7 days after being imaged at the awake condition. Isoflurane gas (2%) was then delivered to the animal through a nose cone in the magnet to maintain the anesthetized state. The body temperature of the animal was monitored and maintained at 37°C ± 0.5°C using a feedback controlled heating pad. All experiments were carried out on a Bruker 4.7T/40cm horizontal magnet (Oxford, UK) interfaced with a Biospec Bruker console. For each session, gradient-echo images covering the whole brain were then acquired using the echo-planar imaging (EPI) sequence with the following parameters: TR = 1s, TE = 30ms, flip angle = 60°, matrix size = 64×64, FOV = 3.2cm×3.2cm, slice number=18, slice thickness = 1mm. 200 volumes were acquired for each run, and six runs were obtained for each session. Functional connectivity was evaluated using seed-based correlational analysis on a voxel-by-voxel basis[4].

Results Figure 1 showed the RSFC maps from the seed of IL. Anticorrelated functional connectivity between IL and amygdala was evident without any global signal correction (referred to as "uncorrected" hereafter) (Fig 1a). Interestingly, negative RSFC from IL was only observed in amygdala while positive RSFC was widely spread across cortical and subcortical areas. With the correction of the global signal (Fig 1c), the spatial location of anticorrelation remained in amygdala. By contrast, the wide spread positive RSFC seen in the uncorrected map was greatly confined to more anatomically specific regions including anterior cingulate cortex (ACC), septum, caudate-putamen (CPU), neuclus accumbens (NAcc) and dorsal lateral prefrontal cortex (dlPFC). The RSFC map obtained after removing the white matter and ventricle signal (Fig. 1b) showed an intermediate pattern between the uncorrected map (Fig. 1a) and the map corrected for the global signal (Fig. 1c). The reciprocal anticorrelated relationship between the amygdala and IL can be observed in the RSFC maps from the amygdala as shown in Figure 2. Negative RSFC was clearly observed in IL in the uncorrected map (Fig. 2a). Similarly, corrections of the ventricle and white matter signal (Fig. 2b) as well as the global signal (Fig. 2c) significantly improved the spatial specificity of positive RSFC between amygdala and HT. The anticorrelation between amygdala and IL remained largely the same. Figure 1 and 2 collectively showed high anatomical specificity of the reciprocal anticorrelated relationship between the amygdala and IL. Considering that one major function of the IL-amygdala circuitry is regulating affective behaviors, it can be expected that anesthesia will disrupt the functional connectivity within the IL-amygdala circuit. Indeed, our data showed that the anticorrelated relationship between IL and amygdala observed in awake rats was completely abolished in isoflurane-anesthetized rats even with the global signal correction as shown in both Fig 1d and Fig 2d. This remarkable difference indicated that: i) the anticorrelated relationship observed in awake rats was not induced by preprocessing methods because the same preprocessing methods were applied to both awake and anesthetized rats data, and ii) the anticorrelation between amygdala and IL has important functional relevance that is impacted by anesthesia.

Conclusion In the present study we have characterized the anticorrelated temporal relationship between spontaneous BOLD fluctuations in IL and amygdala in awake rats. Independent of preprocessing methods, we observed robust anticorrelation within this anatomically well-defined frontolimbic circuit. In addition, this anticorrelation was between two distinct and distant anatomical regions with high anatomical specificity. Furthermore, the anticorrelated relationship between the two regions was absent in anesthetized rats even with global signal regression. Taken together, data of the present study have provided strong evidence validating the existence of anticorrelated RSFC.

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References: **1.**Murphy et al., 2009. **2.** Fox et al., 2005. **3.** King et al., 2005. **4.** Zhang et al., 2010. **5.** Liang et al., 2011. **6.** Liang et al., 2011.

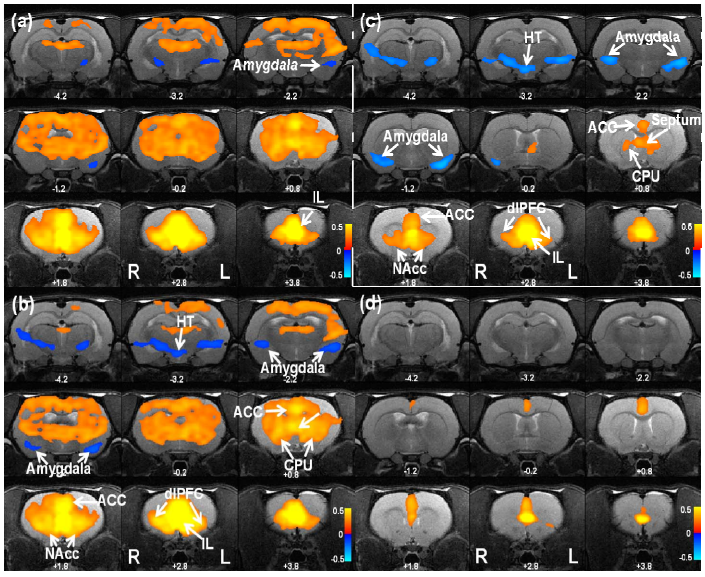

Figure 1. IL RSFC maps. (a) The IL RSFC map in the awake condition without correction of any global signal. (b) The IL RSFC map in the awake condition with correction of the ventricle and white matter signal. (c) The IL RSFC map in the awake condition with correction of the global signal. (d) The IL RSFC map in the anesthetized condition with correction of the global signal. Data from all maps were corrected for six movement parameters. All maps were overlaid on anatomical images. Distances to Bregma (mm) are labeled at the bottom of each image.

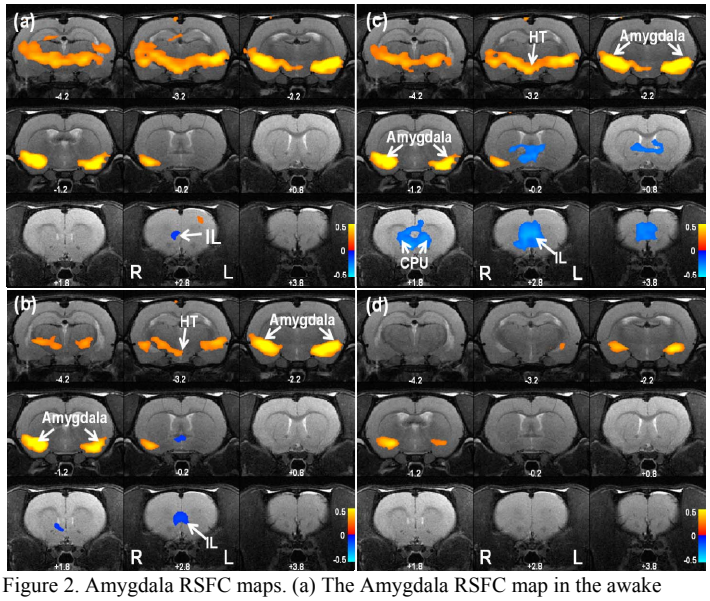

Figure 2. Amygdala RSFC maps. (a) The Amygdala RSFC map in the awake condition without correction of any global signal. (b) The Amygdala RSFC map in the awake condition with correction of the ventricle and white matter signal. (c) The Amygdala RSFC map in the awake condition with correction of the global signal. (d) The Amygdala RSFC map in the anesthetized condition with correction of the global signal. Data from all maps were corrected for six movement parameters. All maps were overlaid on anatomical images. Distances to Bregma (mm) are labeled at the bottom of each image.