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Dynamic resting state functional connectivity in awake and anesthetized rodents

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ABSTRACT

Since its introduction, resting-state functional magnetic resonance imaging (rsfMRI) has been a powerful tool for investigating functional neural networks in both normal and pathological conditions. When measuring resting-state functional connectivity (RSFC), most rsfMRI approaches do not consider its temporal variations and thus only provide the averaged RSFC over the scan time. Recently, there has been a surge of interest to investigate the dynamic characteristics of RSFC in humans, and promising results have been yielded. However, our knowledge regarding the dynamic RSFC in animals remains sparse. In the present study we utilized the single-volume co-activation method to systematically study the dynamic properties of RSFC within the networks of infralimbic cortex (IL) and primary somatosensory cortex (S1) in both awake and anesthetized rats. Our data showed that both IL and S1 networks could be decomposed into several spatially reproducible but temporally changing co-activation patterns (CAPs), suggesting that dynamic RSFC was indeed a characteristic feature in rodents. In addition, we demonstrated that anesthesia profoundly impacted the dynamic RSFC of neural circuits subserving cognitive and emotional functions but had less effects on sensorimotor systems. Finally, we examined the temporal characteristics of each CAP, and found that individual CAPs exhibited consistent temporal evolution patterns. Together, these results suggest that dynamic RSFC might be a general phenomenon in vertebrate animals. In addition, this study has paved the way for further understanding the alterations of dynamic RSFC in animal models of brain disorders.

Introduction

Resting-state functional magnetic resonance imaging (rsfMRI) has revolutionized our understanding of many aspects of human brain networks including their intrinsic functional organizations (Fox et al., 2005; Greicius et al., 2003; Raichle and Snyder, 2007; Wang et al., 2010), developmental and aging profiles (Dosenbach et al., 2010; Pizoli et al., 2011; Smyser et al., 2011; Stevens et al., 2008), and even their genetic basis (Fornito et al., 2011; Hahn et al., 2012; Wiggins et al., 2012). In addition, the plasticity of specific neural circuitries induced by neurobiological behaviors such as learning and memory has been repeatedly revealed by rsfMRI (Albert et al., 2009; Foster and Wilson, 2006; Horovitz et al., 2009). Importantly, alterations in resting-state functional connectivity (RSFC) measured by rsfMRI are tightly linked to numerous neurological, psychiatric and neurodegenerative disorders (Anand et al., 2005; Carter et al., 2012; Greicius et al., 2007, 2004; Hunter et al., 2012; Kennedy et al., 2006; Lowe et al., 2002; Lustig et al., 2003; Mayer et al., 2011; Tian et al., 2006; van Meer et al., 2012; Whitfield-Gabrieli et al., 2009), suggesting that RSFC can potentially serve as a biomarker for aiding diagnosis and evaluating treatment options for brain diseases (Hong et al., 2009).

Until recently, most rsfMRI approaches measure RSFC by detecting the temporal correlations of spontaneously fluctuating rsfMRI signals (Biswal et al., 1995; Calhoun et al., 2001). These approaches implicitly assume that RSFC was stationary during data acquisitions. Since they do not consider temporal variations of RSFC, in essence they only provide an average of functional connectivity over the recording period. Consequently, important dynamic information may be overlooked with the use of such approaches. Indeed, it has been increasingly recognized that RSFC is dynamic in nature (Allen et al., 2014; Chang and Glover, 2010; Grigg and Grady, 2010; Hutchison et al., 2013b; Keilholz et al., 2013; Magri et al., 2012; Pan et al., 2013; Petridou et al., 2013; Thompson et al., 2013, 2014). For instance, Chang and Glover elegantly showed that the coherence and phase of functional connectivity between posterior cingulate cortex and the rest of the default mode network varied over time, clearly demonstrating the non-stationary feature of RSFC (Chang and Glover, 2010). In addition, with clustering analysis human brain networks displayed dynamic but quasi-stable connectivity patterns that diverged substantially from the averaged

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connectivity pattern (Allen et al., 2014). Importantly, simultaneous electrophysiological and fMRI recordings indicated that time-varying RSFC has neurophysiological origin (Chang et al., 2013; Keilholz, 2014; Magri et al., 2012; Pan et al., 2013; Tagliazucchi et al., 2012b; Thompson et al., 2013, 2014).

In parallel with promising results of dynamic RSFC yielded in humans, important findings have also been generated in animals. Using a sliding window approach, Hutchison and colleagues observed that RSFC could vary from strongly positive to strongly negative in anesthetized non-human primates, suggesting that dynamic functional connectivity is a fundamental property in the mammalian brain and cannot be solely attributed to conscious brain processes (Hutchison et al., 2013b). Majeed et al. also observed spatiotemporal dynamic patterns in the rsfMRI data of anesthetized rats (Majeed et al., 2011, 2009). Furthermore, recent work by two different groups (Magri et al., 2012; Thompson et al., 2014) demonstrated a relationship between neural activity and spontaneous fluctuations of BOLD signals similar to the one observed in humans (Chang et al., 2013; Tagliazucchi et al., 2012b). Nevertheless, since all these studies were conducted in anesthetized animals, it is difficult to determine the effects of anesthesia on the dynamic RSFC observed. Therefore, in order to bridge the gap between studies in anesthetized animals and awake humans, it is important to study dynamic RSFC in awake animals.

In the present study we employed the single volume co-activation method (Liu and Duyn, 2013) to systematically study the dynamic features of RSFC in awake and anesthetized rodents. This method, based on the notion that spontaneous fluctuations of BOLD signals might be driven by discrete neural events (Petridou et al., 2013; Tagliazucchi et al., 2012a), selects individual rsfMRI frames with the highest seed signal intensity and decomposes these frames into spatially repeatable co-activation patterns (CAPs). With this method we examined the dynamic RSFC in two neural networks—the somatosensory cortex network which is related to the sensorimotor function, and the medial prefrontal cortex network which is involved in cognitive and emotional functions—in both awake and anesthetized rats. In addition, we extended the single volume co-activation method to investigate the temporal characteristics of dynamic RSFC in rodents.

Materials and methods

Whole brain-coverage rsfMRI data from 42 male Long Evan (LE) rats were acquired in previous studies (Liang et al., 2011, 2012b), and re-analyzed for the purpose of this study. Detailed description of experimental procedures can be found in aforementioned studies. Briefly, all rats were acclimated to the MRI environment and imaging acoustic noise for 7 days as previously described (Liang et al., 2011, 2012a,b; Liang et al., 2013; Zhang et al., 2010) to minimize imaging-related motion and stress. For the imaging setup, rats were briefly anesthetized (2% isoflurane) and secured into a head restrainer with a built-in coil and a body tube. Isoflurane was then discontinued and the whole system was placed into the magnet. Rats were fully awake during imaging sessions. 16 out of 42 rats were also scanned under the anesthetized condition (1.5% isoflurane delivered through a nose cone), which were conducted at least 7 days apart from awake imaging sessions. During anesthetized sessions, the body temperature of the animal was monitored and maintained at 37 °C ± 0.5 °C.

All MRI experiments were conducted on a Bruker 4.7 T magnet with a dual ^{1}H radiofrequency coil configuration (Insight NeuroImaging Systems, Worcester, MA) consisting of a volume coil for excitation and a surface coil for receiving MRI signals. For each MRI session, RARE sequence was used to acquire anatomical images with the following parameters: TR = 2125 ms, TE = 50 ms, matrix size = 256 × 256, FOV = 3.2 cm × 3.2 cm, slice number = 18, slice thickness = 1 mm, and RARE factor = 8. Gradient-echo images were then acquired using the echo-planar imaging (EPI) sequence with the following parameters: TR = 1 s, TE = 30 ms, flip angle = 60°, matrix size = 64 × 64, FOV = 3.2 cm × 3.2 cm, slice number = 18, and slice thickness = 1 mm. 200 volumes were acquired for each rsfMRI run, and six to nine runs were obtained for each session.

The first 10 volumes of each run were removed to ensure the magnetization to reach steady state. rsfMRI images were preprocessed with conventional procedures: registration to a segmented rat brain atlas with MIVA (http://ccni.wpi.edu/), motion correction with SPM8 (Wellcome Department of Cognitive Neurology, London, UK), spatial smoothing (FWHM = 1 mm), regressions of motion parameters and white matter/ventricle signals, band-pass filtering (0.002–0.1 Hz). rsfMRI runs with excessive motion (maximum within-scan displacement >0.5 mm) were discarded. For each run, the blood-oxygenation-level dependent (BOLD) signal was mean-removed and normalized by its standard deviation for each voxel. The seed of bilateral infralimbic cortex (IL) was anatomically defined (17 voxels) based on the segmented rat brain atlas in MIVA, and the seed of unilateral primary somatosensory cortex barrel field (S1BF) was manually drawn (12 voxels) based on the Swanson rat atlas (Swanson, 2004). For each seed, the regionally averaged time series was extracted as the reference time course, and rsfMRI frames with the highest 15% BOLD signal intensity in the reference time course were selected. These steps were carried out in individual runs. Selected frames were concatenated, and then averaged to generate the grand-mean CAP for the seed. Subsequently, concatenated frames were clustered into individual CAPs using k-means clustering. To improve the signal-to-noise ratio, we adopted the thresholding method described in Liu et al. prior to the clustering procedure (Liu and Duyn, 2013). Only the top 15% most activated voxels and the bottom 5% most deactivated voxels with a cluster size of minimal 8 voxels were retained for each frame before entering k-means clustering. The k-means clustering was performed using spatial correlation as the distance measure with 100 replications. We evaluated the quality of clustering using the averaged silhouette values. The result showed that the choice of 3 clusters had the second highest averaged silhouette value, just slightly lower than the choice of 2 clusters (0.0152 for 2 clusters, 0.0129 for 3 clusters, 0.0115 for 6 clusters and 0.0097 for 10 clusters). In light of the study by Liu et al. (2014), the number of clusters was set at 3 (please see more discussion on this issue in Discussion). Frames within each cluster were averaged to obtain the mean CAP for the cluster. The emergence rate of each CAP was calculated as the ratio between the number of frames in the cluster and the total number of selected frames (15% of all frames) for each seed. To evaluate the homogeneity of each cluster, the index of within-cluster similarity was calculated as the averaged spatial correlation coefficient between the cluster-wise mean CAP and individual frames within the cluster.

To further explore the temporal properties of individual CAPs, each cluster-mean CAP map was used as a spatial template, and the spatial correlation coefficients (SCCs) between each individual rsfMRI frame and the template was calculated using Pearson correlation, resulting in a time series of SCC (i.e. r values) for the CAP. Before calculating the spatial correlation, rsfMRI frames were thresholded using the same method mentioned above (Liu and Duyn, 2013). Subsequently, local SCC peaks were identified by the following steps: 1) the total number of selected peak frames (N_{total}) was set at 2% of all frames, 2) the number of selected peak frames for each cluster ($N_{cluster}$) was set at N_{total} × emergence rate of the cluster, 3) $N_{cluster}$ frames with the highest SCC in each cluster were selected as peak frames for the cluster, and 4) if one frame was selected as a peak in multiple clusters, it was assigned to the cluster with the highest SCC. Each local peak frame was defined as Time 0 (t = 0) for an epoch, which also included 5 frames before and 10 frames after t = 0. All epochs of the same CAP were time-lock averaged to reveal the temporal evolution pattern of the CAP. To assess the relative temporal consistency among these epochs, the variance (S.E.M.) at each time point of synchronized epochs for the same CAP was calculated.

To evaluate the effects of motion on dynamic RSFC, a correlation analysis was performed between the volume-to-volume displacement