Uncovering Intrinsic Connectional Architecture of Functional Networks in Awake Rat Brain

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Introduction The effort to understand the connectional architecture of the brain has benefited tremendously from the advent of resting-state functional magnetic resonance imaging (rsfMRI). Using this technique, resting-state functional connectivity (RSFC) as well as global brain networks have been extensively studied. For instance, using graph-based analysis separately identified brain networks sub-serving different functions in humans were found to topologically organize in a non-trivial manner to support efficient information processing. Graph theoretical approaches in rsfMRI uses anatomically or functionally defined regions of interest (ROIs) as 'vertices', and connectivity between ROIs as 'edges'. These approaches have revealed that the human brain's networks are characterized by properties of small-world topology, highly connected hub and high modularity[1]. To date, the majority of studies on intrinsic connectional organization of the brain are conducted in humans. Systematic investigations of this issue in different animal models have been significantly underexplored, partially attributed to confounding effects of anesthetic agent used in animal studies on RSFC. Consequently, it is necessary to explore RSFC in *awake animal*s because it can not only provide invaluable information regarding intrinsic connectional architecture of the animal brain and its reconfiguration in response to cognitive and emotional stimuli, but also may provide a unique window to explore comparative functional anatomy between species. Moreover, understanding connectional architecture in animals will allow us to investigate multiple psychiatric and neurological diseases using translational models. Recently, we have successfully demonstrated the feasibility of mapping RSFC in awake rats[2] based on an *awake animal imaging model* [3]. Using the same animal model here we have characterized the intrinsic network architecture in the awake rat.

Method Sixteen 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 [4-5]. 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. Group independent component analysis (ICA) was applied to the RSFC data using the GIFT toolbox[5] to extract elementary functional clusters of the brain. The connectional relationships between these clusters evaluated by partial correlation analysis were then used to construct a graph of whole-brain neural network. The number of components was set at 40. Brain graphs with all components as 'vertices' and inter-component connectivity as 'edges' were generated and further analyzed based on graph theory to reveal the intrinsic connectional architecture of the rat brain.

Results Fig. 1 showed eight (out of 40) representative components located in specific anatomical regions. Inter-component connections between 40 components were evaluated by partial correlation. Statistical comparison at the group level revealed the pattern of direct connections between different RSFC clusters (one sample t-test, $p < 0.01$) as demonstrated using a graph in Fig. 2a. The total edge number was 78, yielding the connection density of 5.55%. The spectral partitioning algorithm based on the leading eigenvector was applied to this graph and revealed that the rat whole-brain network was segregated into three modules to achieve maximum modularity (Q = 0.414, Fig. 2b-d). This modularity value was significantly higher than both random networks with same nodes and edges and random networks with same degree distribution ($p<0.01$ for both types of random networks), suggesting a prominent modular structure of intrinsic connectional architecture of the rat brain. Of the three modules, module 1 was dominated by cortical regions including the dorsal olfactory bulb, motor cortex, somatosensory cortex, insular cortex and visual cortex as shown in Fig 2b, indicating strong 'direct' communications across the cortical ribbon in the rat[2]. Module 2 included the olfactory system, PFC, ACC, CPu, posterior somatosensory cortex, thalamus, hypothalamus, hippocampus and auditory cortex. This module highlighted the integration of sensory input, cognitive processing and output. Module 3 consisted of the PFC, insular cortex, amygdala, hypothalamus and auditory cortex. This module might be related to emotion and autonomic regulation in the conscious rat. Furthermore, the connectional architecture of the rat brain showed typical features of small-worldness characterized by high clustering coefficient and short minimum path length. When comparing to pure random networks with the same numbers of nodes and edges, the ratio of clustering coefficient (C/C_{random}) was 1.7 and the ratio of minimum path length (L/L_{random}) is 1.08, indicating a higher level of clustering and a similar minimum path length than pure randomized networks. The ratios of these two metrics compared to a random network with the same distribution of degrees showed similar results, C/C_{random}=1.5, and L/L_{random}=1.02. These comparisons collectively suggest that the rat brain is a small-world network.

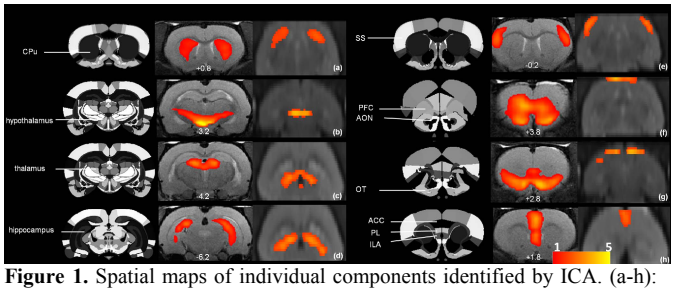

Figure 1. Spatial maps of individual components identified by ICA. (a-h): Examples of ICA components. Left columns are atlas images. Anatomic regions corresponding to individual ICA components are annotated. Middle columns are individual ICA components overlaid on anatomical images in the coronal view. Distances to Bregma (mm) are labeled at the bottom of each image. Right columns are individual ICA components overlaid on anatomical images in the transversal view.

Conclusion In this study, RSFC in awake rats was decomposed into 40 spatial components using group ICA. The direct connectional relationships between these components were evaluated using partial correlation, revealing a complex network linking different regions across the whole brain. This brain network was characterized by the features of small-worldness and significant community structure.

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References: **1.** Bullmore and Sporns, 2009. **2.** Zhang et al., 2010. **3.** King et al., 2005.**4.** Liang et al., 2011. **5.** Calhoun et al., 2001.

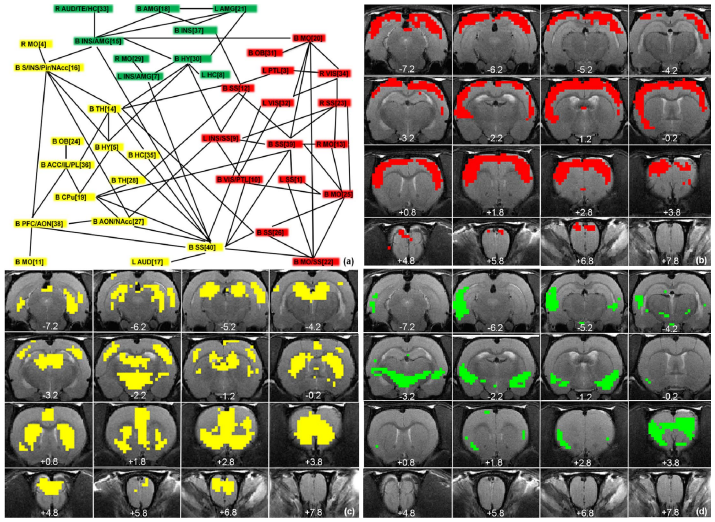

Figure 2. Segregation of the whole-brain network of the awake rat brain. (a). The global functional network constructed based on significant inter-component connections. Each node represents an ICA component labeled with its corresponding anatomy and the ICA number. Each edge represents a significant connection between two components. Nodes within the same module are displayed in the same color (red, green and yellow). Three modules were obtained by the spectral partitioning algorithm. Abbreviations: B, bilateral; L, left; R, right. ACC, anterior cingulate cortex; AMG, amygdala; AON, anterior olfactory nucleus; CPu, caudate-putamen; INS, insula; NAcc, nucleus accumbens; MO, motor cortex; HC, hippocampus; HY, hypothalamus; OB, olfactory bulb; PFC, prefrontal cortex; Pir, piriform cortex; PTL, parietal cortex; S, septum; SS, somatosensory cortex; TE, temporal cortex; TH, thalamus; VIS, visual cortex. (b-d). Community structures of the whole-brain network revealed by spectral partitioning. (b). The first module is dominated by cortical ribbon. (c) The second module is highlighted by the olfactory pathway and its interaction with PFC, and the integration of other sensory input, cognitive processing and output in cortical and subcortical regions like thalamus and hippocampus. (d) The third module includes regions important for emotional and autonomic functions such as amygdala, insular cortex, PFC and hypothalamus. The same colors are used in (b), (c) and (d) as those in (a). Distance to Bregma (mm) is labeled at the bottom of each image.