FUNCTIONAL CONNECTIVITY HUBS AND MODULES IN RESTING-STATE RAT BRAIN

Dany V. D'Souza[1], Elisabeth Jonckers[1], Andreas Bruns[2], Basil Kuennecke[2], Marleen Verhoye[1], Markus von Kienlin[2], Annemie van der Linden[1], and Thomas Mueggler[2]
[1]*Bio-Imaging Lab, University of Antwerp, Wilrijk, Antwerp, Belgium,* [2]*Translational Neuroscience, CNS, Roche, Switzerland*

Introduction: Resting state fMRI (rs-fMRI) is widely used to investigate the brain's intrinsic organizational structure in humans (for review see [1]) and is currently evaluated for its diagnostic and potentially prognostic value in elucidating the relationship between abnormal functional connectivity networks (FCN) and neuropsychiatric disorders such as Alzheimer's disease, schizophrenia and autism [2]. Translation of rs-fMRI applications to small animals has experienced growing interest and FCNs are now increasingly being explored [3-6]. Rs-fMRI bears a great potential in pre-clinical imaging as it allows the topological organization of FCNs in rodent models of various diseases to be assess non-invasively, thus aiding in the development of new therapies. Graph theoretical analysis has recently gained attention for the characterization of FCNs [7, 8]; here, a complex FCN is treated as a graph of nodes and links, wherein the brain regions represent the nodes, and the connections in-between reflect the degree of correlation in their rs-fMRI responses. Two important graph measures that help quantifying the global and local properties of the FCNs are measures of modularity and centrality of brain regions. However, until recently, these measures were mostly applied to FCNs that retained only the strongest positive connections (binary or weighted networks). As the functional significance of negative connections in FCNs becomes evident [9,10], it would be essential to retain these connections for the analysis using a graph approach. In this study, we performed rs-fMRI experiments in rats, and using recently published graph measures [11] we demonstrate for the first time that fully connected, positively and negatively weighted rat FCNs can be segregated into modules connected by hub regions which putatively form a functional core.

Methods: Rs-fMRI experiments were conducted in Sprague Dawley rats (n=5). Body temperature was maintained at 37.0 ± 0.5 °C. Breathing and heart rate, and blood oxygen saturation were monitored during the experiments. Anesthesia during handling comprised isoflurane (2%), administered in a mixture of 30% O_2 and 70% N_2. During the rs-fMRI experiment, animals were sedated using medetomidine (0.1 mg/kg/h; Domitor, Pfizer, Germany). All experiments were performed on a 9.4 T/20 cm BioSpec scanner (Bruker, Germany) using a Bruker linear transmit volume coil and a parallel receive surface array designed for rat head MRI. For each session, T2-weighted images were acquired using a Turbo-RARE sequence (TR/TE=2500/33 ms; 256x256 matrix; 30x30 mm^2 FOV; 12 axial slices; 1 mm thickness). Rs-fMRI data were acquired using a T2*-weighted single shot gradient echo EPI sequence (TR/TE = 2000/16 ms; 128x128 matrix; 30x30 mm^2 FOV; 12 axial slices; 1 mm thickness). 150 EPI volumes were acquired in each run; four runs per subject were available in this study. Pre-processing of rs-fMRI data included rigid-body realignment, spatial normalization of EPI scans to a reference scan (rat #4), in-plane spatial smoothing (0.4x0.4 mm^2), linear regression against rigid-body realignment parameters, band-pass filtering (0.01<f<0.1 Hz). To determine the spatial locations of ROIs, we first manually co-registered the EPI images of *rat #4* to the anatomical RARE images acquired in the same session. Next, ROI masks corresponding to 30 cortical and subcortical brain regions were manually defined bilaterally on the RARE images by referring to the Paxinos atlas [12]. These masks were then resampled and tailored to encompass brain regions on the EPI datasets of all subjects, and were subsequently used in the FC analysis. Partial correlation coefficients of the rs-fMRI time courses were computed between all pairs of brain regions, and subjected to Fisher's z-transform for normality, before averaging across runs and subjects; this resulted in a mean FC matrix, representing a fully-connected weighted graph, with brain regions representing graph nodes, and edges between them reflecting degree of correlation in in their response profiles. We used the following novel network modularity and two centrality measures proposed by Rubinov et al. [11] to characterize both local and global properties of the FCN: a) *Modularity measure (Q*)* estimates the goodness with which functional networks can be partitioned into subgroups, b) *Strength* of the node represents the total weight of connections of a node, c) *Diversity* of a node represents the extent of its inter-modular connectivity. All the above measures were generalized in order to be applicable for fully-connected, positively and negatively weighted FCNs.

Results: The modularity measure partitioned the rat FCN into six modules, with a maximum modularity index of $Q^* = 0.29$ (Fig. 1). Module I included the visual, auditory, retrosplenial cortical areas, and the septum. Module II comprised somatosensory, motor cortex, parietal association area, as well as the hypothalamus. Medial prefrontal (prelimbic) and cingulate cortices were grouped in Module III. Module IV comprised only two brain regions: thalamus and the hippocampus. The largest Module V embraced both cortical (orbitofrontal, insular, entorhinal, and piriform cortex) and subcortical structures (caudate putamen, nucleus accumbens, bed nucleus of stria terminalis, global pallidus, olfactory tubercle, and the amygdala). Finally, Module VI comprised medial geniculate nucleus, parts of mid brain, substantia nigra, and ventral tegmental area. We observed some brain regions with stronger average connection strengths than the rest (Fig. 2a); these high strength nodes included both cortical and subcortical structures. To further distinguish the role of individual nodes in terms of their intra- and inter-modular connectivity we measured the diversity coefficients (Fig. 2b). Regions with simultaneously above mean strength and diversity were classified as network hubs (motor, visual and cingulate cortices, parietal association area, hippocampus, thalamus, and caudate putamen).

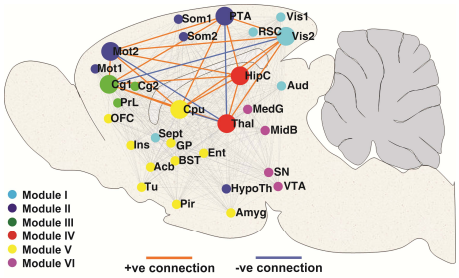

Fig. 1: *Modular organization of the rat FCN. Six modules were identified and are represented by different colors.* ▶ *Network hubs are depicted by larger circles, and functional connections between them are shown by orange (+ve connections) and blue (–ve connections) lines, respectively.*

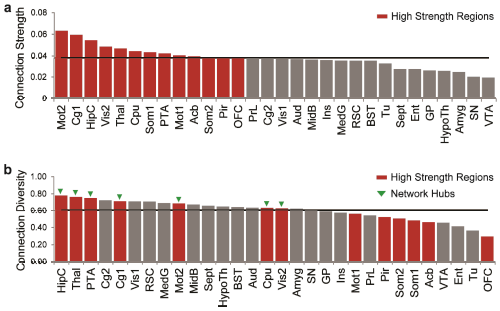

Fig.2: *FCN node characteristics. a) Brain regions are displayed in decreasing order of their generalized connection strength as determined by both positive and negative connections. Strong nodes (strength>mean) are shown in red bars; horizontal line marks the mean strength. b) Brain regions are displayed in decreasing order of their generalized diversity value. Red bars show strong nodes (as determined in Fig. 2a), Strong nodes were further classified as 'network hubs' (green triangles), provided their diversity value exceeded the mean diversity (horizontal line).* ▶

Discussion: To our knowledge, this is the first graph-based study to report on the topological architecture of a fully connected, positively and negatively weighted resting-state FCN in rats. The rat FCN exhibits prominent community structures associated with known brain functions. Module I is relevant for integration of sensory information, Module II has sensory-motoric functions, Module III shows features of default mode networks as observed in humans [13], Module IV is critical for spatial memory [5], Module V represents connections between basal ganglia and cortical areas, which are critical for behavior, and finally Module VI may play a role in locomotion or hearing. The network hubs might play an important role in input and output and form a functional core for information integration within the FCN. This approach offers a promising perspective to demonstrate differences of functional network properties in pharmacological and/or genetic models of neuropsychiatric disorders.

References: [1] Van den Heuvel et al. *Eur Neuropsychopharmacol* (2010). [2] Fox et al. *Front. Syst. Neurosci* (2010). [3] Hutchison et al. *J Neurophyiol* (2010). [4] Liang et al. *J Neurosci* (2011). [5] Becerra et al. *PLoS One* (2011). [6] Jonckers et al. *PLoS One* (2011). [7] Rubinov et al. *NeuroImage* (2010). [8] Wang et al. *Front. Syst. Neurosci* (2010). [9] Liang et al. *NeuroImage* (2011). [10] Chai et al. *NeuroImage* (2011). [11] Rubinov et al. *NeuroImage* (2011). [12] Paxinos and Watson. *The Brain in Stereotaxic Coordinates* (Els. Acad. Press. 2005. 5th Edn.). [13] Damoiseaux et al. *Cereb. Cortex* (2006).