Mapping the Resting State Functional Connectivity in the GPR88 KO mouse brain

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Introduction:

Resting state functional MRI (rsfMRI) developed during the last decade as a powerful tool for non-invasive explorations of brain functional connectivity (FC)[1, 2]. The resting state functional networks were consistently mapped in the human brain and their reorganization underlying various neurologic and psychiatric disorders was demonstrated. The pathophysiology of many psychiatric illnesses, including depression, bipolar disorder, schizophrenia or attention deficit hyperactivity disorder are taught to involve altered brain circuitry and function of specific brain regions. The molecular mechanisms underlying such alterations are incompletely understood. The orphan G protein-coupled receptor (GPCR) "GPR88", draw increasing attention in the clinical and pre-clinical psychiatric research, because its expression was shown to be modulated by several psychiatric disorder-related treatments[3, 4]. However, the neurobiological function and of GPR88 and the impact of its modified expression on brain FC are - so far - unknown. In this context, the aim of our study was to investigate the role of GPR88 in brain functional communication. We non-invasively probed using rsfMRI, independent component analysis (ICA) and graph theory the intrinsic connectional architecture of functional networks in GPR88 Knock Out (KO) mice.

Materials and Methods:

8 weeks old wild type (n=15, control group) and GPR88-/- (n=15, KO group) male (74.9% C57B/6J, 25% 129/SvPas, 0.05% FVB/N, 0.05% SJL/J) mice were imaged using a 7T small bore animal scanner (Biospec 70/20, Bruker, Germany) and a mouse brain adapted CryoCoil (Bruker, Germany), under an optimum sedation protocol using Medetomidine (MD) [initial subcutaneous (s.c.) bolus of 0.3 mg MD/kg BW, followed by a continuous s.c. infusion (0.6 mg MD/kg BW, 200 µl/hour)]. The physiological conditions (body temperature, respiration, heart rate, blood oxygen saturation) were monitored throughout the scanning session. rsfMRI data was acquired with T_2* - weighted single shot GE-EPI sequences (TE/TR = 10 ms/1700 ms). The mouse brain (excluding the cerebellum) was covered using 12 axial slices of 0.7 mm thickness, with a field of view of 19.2 X 12 mm^2 and a planar resolution of 150 X 150 μm^2. 200 volumes were recorded in interlaced fashion for each run. *Pre-processing of the rsfMRI data* was done using SPM8 for motion correction, spatial normalization and alignment to an anatomical brain template and smoothing (Gaussian kernel of FWHM of 0.4x0.4x1mm^3). Group spatial Independent Component Analysis (ICA) using the MATLAB tool GIFT[5] was carried out on combined rsfMRI data sets using ICASSO (with 20 runs) to evaluate the reliability[6] of each identified component. The number of components was set at 100 and the spatial maps of the independent components (IC) were scaled to z scores. The direct connectivity between each pair of IC was further assessed via partial *Pearson Correlation* (PC) of their time courses. This resulted into two PC matrices, averaged across the control and KO group. These graphs represented the ICA components (brain regions) as nodes[7] and assigned the degree of correlation in their response profile (weight). From this, the strength of each node (average weight of relevant positive connections) was calculated. Focusing on positive correlations only, the PC matrices were converted into binary ones using a $p<0.05$ relevance threshold and the brain regions (nodes) with the highest number of statistically relevant connections were identified. *Graph theoretical approaches* were used to check the partition of mouse brain functional network into "community structures" or modules based on group ICA results. The "diversity" was further calculated[8] to quantify the inter-modular connectivity of each node (IC).

Results and Discussion:

Reliable and stable functional clusters were obtained using 100 component group ICA (ICASSO), located in well-defined brain areas. Figure 1 shows an example of biologically relevant components (from left to right) Amygdala (bilateral) and Caudate Putamen (CPu – UniL and UniR) where GPR88 gene exists[9]. Seed correlation method was used to check the connectivity with those brain regions to others. ICA with bootstrapping ICASSO 20 repetitions resulted in a clustering index $I_q>0.8$ in 91% of the 100 components, indicating a high stability and reproducibility of the resulting patterns. 10 out of 100 components were excluded by visual inspection as being of vascular/ventricular origin or artifactual. The influential brain regions were classified for both groups based on their strength and diversity. Brain regions having strength and diversity above their respective mean value were considered as hubs. Several findings point toward a remodeling of the mouse brain connectivity in the absence of GPR88. Firstly, less and different brain regions were identified as functional connectivity hubs in the GPR88 KO group when compared to the controls. 11 and 7 hubs were found in control and GPR88 KO group respectively. 2 hubs were identical in both groups. Those are: RSG (middle) and RSA (right). Excluding those areas from both groups, figure 2a shows 9 distinct hubs identified in the control group and 2b shows 5 hubs specific for the KO group (abbreviations adapted from Paxinos Mouse Brain Atlas[10]). Moreover, 2 additional modules were obtained in KO group (total no. of modules: 7) compared with the control group (total no. of modules: 5), indicating higher degree of functional network segregation.

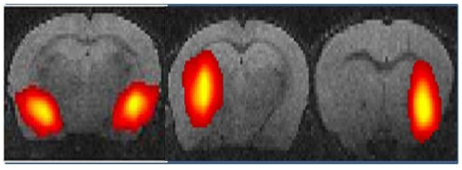

Figure1: Examples of biologically relevant components: Amygdala(Bi) and CPu (L&R)

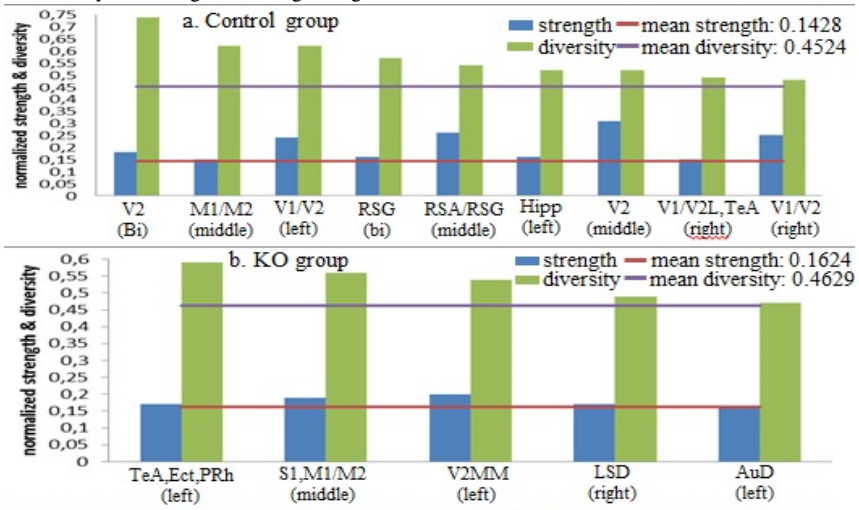

Figure 2: Components with strength and diversity above their respective mean value a) in control group, and b) in KO group. Abbrevaitons adapted from Paxinos Mouse Brain Atlas

Conclusion:

This study demonstrates the potential of rsfMRI to non-invasively probe the brain functional networks in genetically modified mice. Here we have shown preliminary data indicating a remodeling of functional networks in GPR88 KO mice. Further study involving parallel behavioral investigations will expand our understanding about the implication of GPR88 gene on the development of neurological or psychiatric disorders.

References:

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