Remodeled resting state functional connectivity pattern in the default mode network and cortico – striatal circuitry of GPR88 knock-out mouse brain

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Introduction: The role of striatum specific G protein coupled receptor[1] (GPCR) 'GPR88' in brain functional communication is so far unknown even though it's modulated expression in several psychiatric disorder related treatments has been observed[2, 3]. In this present study we investigated the neurobiological function of GPR88 and the impact of its modified expression on brain functional connectivity (FC) using resting state functional magnetic resonance imaging (rsfMRI) technique. We perceived extensive alterations in the cortical and sub-cortical brain regions of the GPR88 -/- mice, highlighting functional connectivity remodeling in the Default Mode Network (DMN) and cortico – striatal circuitry, involved in several psychiatric illness including parkinson's[4-6], schizophrenia[6], huntington's[7], or attention deficit hyperactivity disorder (ADHD)[8]. Nonetheless, the molecular mechanisms underlying such alterations are incompletely understood. Our intriguing results promote further investigations of the involvement of the GPR88 gene in various psychiatric disorder and hence in the discovery of new therapeutic strategies.

Materials and Methods: 8 weeks old GPR88 +/+ (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[9] was carried out on combined rsfMRI data sets using ICASSO (with 20 runs) to evaluate the reliability[10] 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 GPR88 +/+ and GPR88 -/- group. These graphs represented the ICs (brain regions) as nodes[11] and assigned the degree of correlation in their response profile (weight). To assess the significant correlation differences among the brain regions between groups, we applied False Discovery Rate (FDR) correction, thresholding at $p<0.05$ to the PC matrices. This resulted in the generation of the matrix containing only the significant correlation differences among the brain regions between GPR88 +/+ and GPR88 -/- mice group.

Results and Discussion: Reliable and stable RSNs were obtained using 100 component group ICA (ICASSO), located in well-defined brain areas. ICA with bootstrapping ICASSO 20 repetitions resulted in a clustering index I_q>0.8 in 96% 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. Figure1 shows an example of the resting state FC maps of some components obtained from 100 ICA (from top left to bottom right: left and right lateral nucleus accumbens, bi-lateral amygdala, left lateral caudal and right lateral rostral caudate putamen respectively), highlighting the brain regions where GPR88 gene has the highest expression.

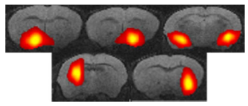

Figure1: RSFC maps of individual components identified by 100 ICA. From top left to bottom right: left and right lateral nucleus accumbens, bi-lateral amygdala, left lateral caudal and right lateral rostral caudate putamen respectively.

Time courses of 90 individual ICs of each experimental group were assigned to specific brain regions and used to evaluate the inter-component connectional relationship with the partial correlation (PC) algorithms. By means of FDR correction algorithm we observed significant alterations of the brain FC patterns in the GPR88 -/- mice. Based on this, 44 out of 90 components were identified as over-averaged significantly altered in correlation (functional connectivity). Figure 2A shows the patterns of some of the ICs highlighting brain regions with significantly modified functional connections (from top left to the bottom right: left-lateral visual cortex - VC, bi-lateral retrosplenial cortex - RSP, left-lateral somatosensory cortex - SSC, right-lateral hypothalamus - Hyp, left-lateral caudate putamen - CPu, left-lateral auditory cortex - AUD, left lateral dorsal insular cortex - IC, bi-lateral motor cortex – MC). We observed massive FC alterations in the cortical and sub-cortical regions like striatum and hypothalamus, highlighting FC remodeling in the cortico – striatal circuitry. Most of the cortical regions, such as: anterior cingulate cortex (ACC), retrosplenial cortex (RSP), auditory cortex (AUD), temporal association cortex (TeA), along with sub-cortical hippocamous (HIPP.) (Figure2B – from left to right), belong to the DMN, posited to play a fundamental role in brain organization[12-14]. Figure2C shows the significant correlation differences among DMN regions between groups. Our results demonstrate significant FC alterations in the DMN and cortico – striatal circuitry involved in several psychiatric illness[4-8].

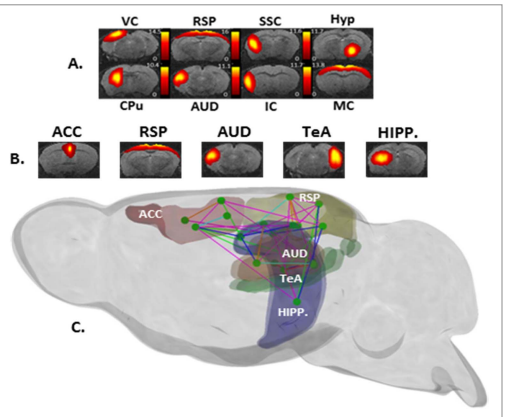

Figure2: A. RSFC maps of the brain regions with significantly modified functional connections (from top left to the bottom right: VC, RSP, SSC, right-lateral hypothalamus - Hyp, CPu, AUD, IC, MC. **B.** RSFC maps of the DMN components (left to right: ACC, RSP, AUD, TeA, and Hipp.). **B.** DMN regions highlighted in different colors overlaid on the sagittal mouse brain template (dark red – ACC, mustard – RSP, brown – AUD, green – TeA, violet – Hippocampus). Six different colored lines show the significant correlation differences among above mentioned DMN regions between GPR88 +/+ and GPR88 -/- group.
Red lines - positive correlation: GPR88 +/+ group > GPR88 -/- group, **Green lines** - positive correlation: GPR88 +/+ group < in the GPR88 -/- group, **Blue lines -** anti-correlation:
GPR88 +/+ group > in the GPR88 -/- group, **Cyan lines -** anti-correlation: GPR88 +/+ group < in the GPR88 -/- group, **Brown lines -** positive correlation in the GPR88 +/+ group but anti-correlation in the GPR88 -/- group, **Magenta lines -** anti-correlation in the GPR88 +/+ group but positive correlation in the GPR88 -/- group

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 cortico-striatal pathway in GPR88 -/- 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.

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