Remodeling of Sensorimotor Brain Connectivity in *Gpr88*-Deficient Mice

Tanzil Mahmud Arefin,^{1–4} Anna E. Mechling,^{1,2} Aura Carole Meirsman,^{5,6} Thomas Bienert,¹
Neele Saskia Hübner,^{1,2} Hsu-Lei Lee,¹ Sami Ben Hamida,^{5,7} Aliza Ehrlich,^{5,7} Dan Roquet,⁸
Jürgen Hennig,¹ Dominik von Elverfeldt,¹ Brigitte Lina Kieffer,^{5,7} and Laura-Adela Harsan^{1,8,9}

Abstract

Recent studies have demonstrated that orchestrated gene activity and expression support synchronous activity of brain networks. However, there is a paucity of information on the consequences of single gene function on overall brain functional organization and connectivity and how this translates at the behavioral level. In this study, we combined mouse mutagenesis with functional and structural magnetic resonance imaging (MRI) to determine whether targeted inactivation of a single gene would modify whole-brain connectivity in live animals. The targeted gene encodes GPR88 (G protein-coupled receptor 88), an orphan G protein-coupled receptor enriched in the striatum and previously linked to behavioral traits relevant to neuropsychiatric disorders. Connectivity analysis of *Gpr88*-deficient mice revealed extensive remodeling of intracortical and cortico-subcortical networks. Most prominent modifications were observed at the level of retrosplenial cortex connectivity, central to the default mode network (DMN) whose alteration is considered a hallmark of many psychiatric conditions. Next, somatosensory and motor cortical networks were most affected. These modifications directly relate to sensorimotor gating deficiency reported in mutant animals and also likely underlie their hyperactivity phenotype. Finally, we identified alterations within hippocampal and dorsal striatum functional connectivity, most relevant to a specific learning deficit that we previously reported in *Gpr88*^{−/−} animals. In addition, amygdala connectivity with cortex and striatum was weakened, perhaps underlying the risk-taking behavior of these animals. This is the first evidence demonstrating that GPR88 activity shapes the mouse brain functional and structural connectome. The concordance between connectivity alterations and behavior deficits observed in *Gpr88*-deficient mice suggests a role for GPR88 in brain communication.

Keywords: default mode network; *Gpr88*; mouse brain functional connectivity

Introduction

NEURONS FORM STRUCTURAL and functional networks that drive brain function and behavior (Van Essen, 2013). Connectome genetics, or the analysis of brain connectivity in relation to gene expression and function, addresses how disease

genes influence brain connectivity in humans (Richiardi et al., 2015; Thompson et al., 2013), and also links gene transcriptional patterns with neural network activities in both humans and mice (Richiardi et al., 2015). These studies, however, remain correlative in nature. Deep understanding of cognitive and behavioral development, adaptation, or dysfunction also

¹Department of Radiology, Medical Physics, Medical Center University of Freiburg, Faculty of Medicine, University of Freiburg, Freiburg, Germany.

²Faculty of Biology, University of Freiburg, Freiburg, Germany.

³Bernstein Center Freiburg, University of Freiburg, Freiburg, Germany.

⁴Bernard and Irene Schwartz Center for Biomedical Imaging, Department of Radiology, New York University School of Medicine, New York, New York.

⁵Département de Médecine Translationnelle et Neurogénétique, Institut de Génétique et de Biologie Moléculaire et Cellulaire, INSERM U-964, CNRS UMR-7104, Université de Strasbourg, Illkirch-Graffenstaden, France.

⁶Neuroscience Paris Seine, Institut de Biologie Paris Seine, CNRS UMR 8246/INSERM U1130/Université Pierre et Marie Curie, Paris, France.

⁷Douglas Mental Health Institute, Department of Psychiatry, McGill University, Montreal, Quebec, Canada.

⁸Engineering Science, Computer Science and Imaging Laboratory (ICube), Integrative Multimodal Imaging in Healthcare, University of Strasbourg—CNRS, Strasbourg, France.

⁹Department of Biophysics and Nuclear Medicine, Faculty of Medicine, University Hospital Strasbourg, Strasbourg, France.

requires adapted approaches to identify molecular and network determinants of healthy and pathological brains. Our recent work, allying targeted mouse mutagenesis and fine-grained magnetic resonance (MR)-based neuroimaging of live animals, revealed a gene-to-network signature for the mu opioid receptor with predominant alteration of pain/aversion networks (Mechling et al., 2016). This proof-of-principle study, based on open-ended whole-brain connectivity analysis, demonstrates the power of combined gene knockout/MRI to decipher consequences of a single gene inactivation on brain networks and potentially predict behavioral outcomes of genetic dysfunction. In this study, we developed a similar approach to tackle the function of the orphan *Gpr88* (G protein-coupled receptor 88) receptor gene, encoding another G protein-coupled receptor whose ligand remains unknown, and discovered brain network mechanisms underlying major GPR88-controlled behaviors.

GPR88 is a striatal-enriched G protein-coupled receptor, expressed in rodents, monkeys, and humans during development and in adulthood (Massart et al., 2016). In humans, the *Gpr88* gene was associated with bipolar disorders and schizophrenia (Del Zompo et al., 2014), and the potential of GPR88 as a target to treat psychiatric disorders has attracted increasing interest. In mice, deletion of the *Gpr88* gene has been studied with a primary focus on striatal-mediated behaviors, and null mutant mice show motor coordination deficits (Logue et al., 2009; Meirsman et al., 2016a; Quintana et al., 2012), reduced prepulse inhibition (Logue et al., 2009), stereotypies (Meirsman et al., 2016a), and modified cue-based learning (Meirsman et al., 2016a; Quintana et al., 2012). Recently, we found that *Gpr88* knockout mice also show improved hippocampal-dependent learning and reduced anxiety levels (Meirsman et al., 2016b). This receptor therefore controls a much larger behavior repertoire than anticipated and, beyond motor activity, also engages spatial learning, emotional processing, and the evaluation of environmental stimulus value. In this study, we examined *Gpr88* knockout mice using combined resting-state functional MR imaging (rsfMRI)/diffusion tensor imaging (DTI) in live animals to identify neural networks depending on *Gpr88* gene activity and used open-ended whole-brain connectivity analysis to determine predominant alterations that would predict major functions of *Gpr88* related to neuropsychiatric conditions.

Materials and Methods

Additional detailed methods are provided in the Supplementary Data (Supplementary Data are available online at www.liebertpub.com/brain).

MRI experiments

Animals. MRI was performed on two groups ($n=14$ /group) of 7–8-week-old live adult male mice (74.9% C57B/6J, 25% 129/SvPas, 0.05% FVB/N, 0.05% SJL/J): wild-type control mice (CTRL: $n=14$) and the *Gpr88* knockout group (*Gpr88*^{−/−}: $n=14$), respectively. All animal experiments were performed in accordance with the guidelines and ethics on animal experimentation established by the German and French laws: ethical allowance 35_9185.81/G-13/15 from Regierungspräsidium Freiburg, Germany, and CREMEAS, 2003-10-08-[1]-58, Strasbourg-France, respectively.

Animal preparation. The animals were briefly anesthetized with isoflurane during imaging preparation (stereotaxic fixation of the mouse head, attachment of physiological monitoring devices). To avoid the inhibitory effects of isoflurane on the blood oxygen level-dependent (BOLD) signal, anesthesia was further switched to medetomidine (MD—Domitor; Pfizer, Karlsruhe, Germany). Moderate MD sedation was initially induced by a subcutaneous (s.c.) bolus injection (0.3 mg MD/kg body weight in 100 μ L 0.9% NaCl solution); 15 min later, the animals received a continuous s.c. infusion of MD through an MRI-compatible catheter (0.6 mg/kg body weight in 200 μ L/h) subcutaneously inserted at the mouse shoulder level. After rsfMRI acquisition, MD infusion was stopped and replaced by anesthesia through isoflurane (~ 1.5 vol%) for further scanning performed on respiration triggering. Isoflurane induces a deeper anesthesia, important for avoiding the movement artifacts for diffusion imaging. Mouse physiological conditions (including temperature, respiration, and blood oxygen saturation) were monitored continually during the imaging session.

MRI data acquisition. Mouse brain MRI data were acquired with a 7 T small-bore animal scanner (Biospec 70/20, Bruker, Germany) and a mouse head adapted CryoCoil (MRI CryoProbe, Bruker, Germany).

- rsfMRI: Data were acquired with T_2^* -weighted, single-shot, gradient-echo echo-planar imaging (GE-EPI) sequence (echo time [TE]/repetition time [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 (FOV) of 19.2×12 mm² and an acquisition matrix of 128×80 , which resulted in a planar resolution of 150×150 μ m². Two hundred volumes were recorded in an interlaced manner for each run.
- T_2 : High-resolution morphological images were acquired using Turbo rapid acquisition with relaxation enhancement (RARE) T_2 sequence (TE/TR=50 ms/6514 ms, two averages at RARE factor of 4). The whole brain, including cerebellum, was covered using 48 slices (0.3 mm slice thickness) at planar spatial resolution of 51×51 μ m² with an FOV of 1.3×1.0 cm² and an acquisition matrix of 256×196 .
- High-angular resolution diffusion imaging (HARDI) was performed using four-shot DTI-EPI sequence. Twenty-five axial slices of 0.5 mm thickness were acquired at a resolution of 94×94 μ m² with an FOV of 1.5×1.2 cm² and an acquisition matrix of 160×128 , covering the equivalent partition of the brain as for the rsfMRI scan (TE/TR=27 ms/3750 ms); $\Delta=10$ ms, diffusion gradient duration (δ)=4 ms, b factor of 1000 s/mm², 30 noncollinear diffusion gradient directions.

MRI data preprocessing. Imaging data were preprocessed (Mechling et al., 2016) using MATLAB (The MathWorks, Natick, MA) along with the fMRI tool of statistical parametric mapping SPM8* and its SPM Mouse[†] toolbox (Sawiak et al., 2013), which includes functions for realignment, coregistration, and segmentation of mouse brain data (see detailed procedure in Supplementary Data: “Data preprocessing”). Briefly, the

*www.fil.ion.ucl.ac.uk/spm

†www.spmmouse.org

preprocessing pipeline included an initial realignment of the 200 volumes of rsfMRI data to the first one using a least square approach and a six-parameter rigid body transformation in space to correct for motion in each single scan. The coregistration function also was used to align the rsfMRI (first time point) volumes of each mouse to its respective A_0 images obtained from HARDI (HARDI acquisition with a diffusion weighting factor $b_{\text{factor}} = 0 \text{ mm}^2/\text{s}$ —no diffusion gradient applied) and T_2 image volume. SPM Mouse brain template was further refined by including additional high-resolution mouse brain images to create a tissue probability map (TPM) template. We used this template for spatial normalization and alignment of rsfMRI mouse brain images, morphological T_2 -weighted images, the A_0 images, and the parametric maps derived from diffusion tractography (fiber density [FD] and fractional anisotropy maps). We applied a Gaussian smoothing with a kernel of full width at half maximum of $0.4 \times 0.4 \times 1 \text{ mm}^3$ to all TPM-aligned rsfMRI image volumes (Mechling et al., 2014). Furthermore, the whole brain was parceled using an in-house-developed MATLAB and Allen Mouse Brain Atlas (AMBA) (Lein et al., 2007). The AMBA was aligned and resliced to our template using the SPM8 toolbox, changing its initial resolution to our template's resolution of $165 \times 230 \times 135$ voxels with a voxel size of $0.07 \times 0.07 \times 0.07 \text{ mm}^3$.

MRI data postprocessing

Resting-state functional magnetic resonance imaging

Independent component analysis. High-dimensional (100 components) spatial group independent component analysis (ICA) (Calhoun et al., 2001) using the MATLAB-based toolbox GIFT (group ICA of fMRI toolbox—v1.3i, www.nitrc.org/projects/gift) was carried out on 28 combined control (CTRL) and *Gpr88*^{-/-} datasets. Infomax algorithm was used to decompose the entire BOLD data set into spatially independent components (ICs) without any hypothesis paradigms (Hyvärinen and Oja, 2000). We further investigated the robustness of the identified components using ICASSO algorithm (Himberg et al., 2004).

Estimation of the number of components is an important step while decomposing the entire BOLD signal into spatially ICs or sources. Underestimation of the components may result in mixing various components (Margulies et al., 2010; van de Ven et al., 2004), whereas overestimation can result in splitting reliable networks (Esposito et al., 2003; Moritz et al., 2005), decreasing the stability of IC estimates (Li et al., 2007). Therefore, we used ICASSO algorithm (Himberg et al., 2004) to assess the stability pattern by bootstrapping and randomizing initial conditions for different numbers of ICs. The quality index I_q (values ranging from 0 to 1) was used as a quantitative measure of robustness of the identified components evaluating compactness and isolation of each cluster (Hübner et al., 2017; Mechling et al., 2014). We verified the consistency of the results when progressively achieving a high spatial definition (in accordance to fine anatomical details) of the functional clustering patterns with 10, 15, 20, 40, 60, 80, 100, and 120-ICASSO, respectively. Using the percentage of components revealing quality index (I_q) > 0.75 as a stability criterion, we observed a clear degradation of the IC estimates for 120-ICASSO, justifying our choice of 100-ICASSO analysis.

The mean resulting patterns were displayed as spatial color-coded z -maps onto T_2 -weighted morphological images (threshold $|z| > 3$, corresponding to $p < 0.00135$) and on coregistered AMBA (Lein et al., 2007). The color coding represents the dependence of the time course in each voxel compared with the mean time course of the respective component in arbitrary units. Coregistration with AMBA allowed for automatic identification of anatomic brain areas covered by IC patterns. From the 100-ICASSO results, 12 artifactual components related to cerebrospinal fluid, vascular, or movement-related pseudo activations were excluded from analysis after visual inspection and overlay on AMBA. From these aggregate components and the original data, we computed spatial, back-reconstructed, individual subject components using a spatial-temporal regression approach (see also Supplementary Data). We further used the back-reconstructed, individual spatial maps to create incidence maps for each IC. Relevant examples are the incidence maps illustrating the patterns of SS, CP, ACB, and AMY functional clusters (Supplementary Fig. S1c; Supplementary Data are available online at www.liebertpub.com/brain). The incidence maps illustrate the spatial distribution and the reproducibility of the IC pattern over each animal group (CTRL and *Gpr88*^{-/-}). The color-coded incidence of a voxel reflects in how many of the animals it was found to belong. These examples show low intragroup variability of ICA patterns and extremely high similarity between group patterns.

The results substantiate our further approach of using the meaningful 88 group ICASSO functional clusters (Supplementary Fig. S2) as nodes in the generation of mouse brain functional connectivity matrices (MBFC) of CTRL and *Gpr88*^{-/-} groups of animals through partial correlation (PC). Conducting the 100-ICASSO separately on each animal group would have eventually resulted in slightly different nodes of connectivity and difficulties to directly compare the group results.

Pearson PC analysis. The PC coefficients (Pearson) between each pair of ICs derived with ICASSO were calculated and used to create an 88×88 adjacency PC matrix for each animal as well as two average matrices, representative for each experimental group (group-specific PC matrices: Fig. 1a). Each element of the matrix represented the strength of direct connectivity between two components (nodes). The PC matrices were then normalized using Fisher's z -transformation. The significance of positive and negative correlations between pairs of components was further assessed by using a two-sided one-sample t -test and thresholding at $p < 0.05$.

Direct intergroup (CTRL vs. *Gpr88*^{-/-}) statistical comparison of the group matrices was further performed. The functional connectivity (FC) alterations between groups were considered significant after assessment using a two-sample t -test ($p < 0.01$; false discovery rate [FDR] corrected). A group comparison matrix (GCM) was generated (Fig. 1b) that color coded the statistically significant intergroup differences of connectivity. GCM was further used to count the significantly changed connections for each node (IC) and we further ranked nodes on the basis of highest number of such statistically significant differences in connectivity across the two genotypes (Fig. 1c).

Seed-based correlation analysis. To evaluate the alterations of functional networks, several brain areas that showed high number of correlation alterations between groups were

FIG. 1. Quantitative mapping of functional network alterations in *Gpr88*^{-/-} mice reveals a strong *Gpr88*-dependent activity signature in live animals: **(a)** Group-specific resting-state FC matrices (CTRL above and *Gpr88*^{-/-} group below the diagonal), including both positive and negative internodal correlations (derived via partial correlation methods). The nodes were defined through functional segregation using 100-ICASSO. Functional nodes were grouped and assigned to corresponding anatomical regions through coregistration on the Allen Mouse Brain Atlas **(d)**. **(b)** Direct intergroup (CTRL vs. *Gpr88*^{-/-}) statistical comparison of connectivity matrices (two-sample *t*-test, $p < 0.01$, FDR corrected) is shown as a 2D matrix. The *Gpr88* genetic inactivation induced widespread modifications of internode connectivity. The red circle points to the cluster of strong intracortical FC remodeling in the absence of *Gpr88*, whereas the red square points to extensive significant FC alterations of the RSP area connectivity. **(c)** Nodes of brain area with highest number of statistically significant connectivity changes are ranked (two-sample *t*-test, $p < 0.01$, FDR corrected). Their functional pattern is overlaid on four T₂-weighted anatomical images. From top to bottom: retrosplenial area (RSP), somatosensory area (SS), motor area (MO), visual area (VIS), thalamus (TH), temporal association area (TEa), superior colliculus (SC), caudate-putamen (CP), agranular insular area (AI), hippocampal formation (HPF), and anterior cingulate area (ACA). **(d)** Assignment of brain regions from rostral to caudal direction and their corresponding localization on the sagittal mouse brain template adapted from the Allen Mouse Brain Atlas. The anatomical overlap of components was identified using a MATLAB-based and in-house-developed postprocessing tool, which is based on coregistration of the data on the Allen Mouse Brain Atlas (AMBA) (Lein et al., 2007) (<http://mouse.brain-map.org/static/atlas>). We assigned the component to the region or anatomical subdivision (in case of large areas) with maximum overlap. Nevertheless, components are partly touching other regions (subdivisions). CTRL, control; FC, functional connectivity; FDR, false discovery rate; GPR88, G protein-coupled receptor 88; RSP, retrosplenial.

selected as the regions of interest (ROIs). As all the data were normalized onto AMBA, each ROI was extracted using this atlas and used as the seed region to perform FC analysis of preprocessed rsfMRI data. Correlation coefficients were then computed (two-tailed t -test, $p < 0.001$) between the seed region and the averaged time series of the remaining whole brain for each group and were converted to z values using Fisher's r -to- z transformation. We further performed voxel-level general linear model ($p < 0.001$) corrected for multiple comparisons using a random field theory approach (Worsley et al., 1996) to statistically evaluate FC remodeling on the group level for each specific seed.

Diffusion-based tractography. Modifications in the brain structural connectivity were assessed using HARDI and further tractography using a global fiber tracking approach (Harsan et al., 2013; Reisert et al., 2011). FD maps were used to measure brain microstructural modifications. Statistically significant differences were evaluated using a two-sample t -test ($p < 0.05$, familywise error [FWE] corrected) (detailed procedure is described in Supplementary Data).

Results

GPR88 expression in the mouse brain

We verified the expression pattern of GPR88 in the control (CTRL) mice by *in situ* hybridization (Supplementary Fig. S1a). We observed the receptor expressed in the layers 4 and 5 of the somatosensory cortex (SS), caudate-putamen (CP), amygdala, nucleus accumbens (ACB), and olfactory tubercle (OT) in support of several previously reported literatures (Becker et al., 2008; Ghate et al., 2007; Massart et al., 2016; Mizushima et al., 2000; Van Waes et al., 2011).

Elementary functional clusters identified through group 100 ICA

We applied high-dimensional, data-driven spatial ICA (using 100 components) combined with ICASSO on rsfMRI datasets from CTRL and *Gpr88*^{-/-} animal groups. Eighty-eight reliable functional clusters were identified whose spatial pattern covered neuroanatomical regions defined by the Allen Mouse Brain Atlas (AMBA) (Supplementary Fig. S2). We further validated the reproducibility of the group ICASSO patterns in each animal and in each experimental group through back reconstruction (see the Materials and Methods section and Supplementary Fig. S1b, c). The results demonstrated low intragroup variability of the component pattern and extremely high similarity between groups. We could associate the spatial pattern of some of these components with areas strongly expressing *Gpr88* receptors in normal conditions (Supplementary Fig. S1b). Both left and right hemispheric patterns were obtained and presented in Supplementary Figure S1 for cortical (SS) and subcortical areas (CP, ACB, and AMY). We further used the 88 group ICASSO functional clusters (Supplementary Fig. S2) as nodes in the generation of resting-state brain FC (rsFC) matrices of CTRL (Fig. 1a—above the diagonal) and *Gpr88*^{-/-} (Fig. 1a—below the diagonal) groups of animals (two-sided one-sample t -test, $p < 0.05$) (see the Materials and Methods section). All these nodes correspond to anatomically well-defined brain regions and were rearranged according to their association with brain areas in the rostrocaudal axis (Fig. 1d).

Deletion of GPR88 receptor induces extensive FC remodeling of the mouse brain

We quantitatively evaluated the impact of GPR88 receptor deletion on the mouse brain FC using direct statistical intergroup comparison of CTRL and *Gpr88*^{-/-} MBFC matrices (see the Materials and Methods section and Fig. 1b). We detected significant and widespread alterations of internode connectivity (Fig. 1b; two-sample t -test, $p < 0.01$, FDR corrected). The 2D matrix representation (Fig. 1b) captured the causal effect of targeted *Gpr88* gene disruption at the level of whole brain networks. The extent of *Gpr88*-dependent connective activity appeared surprisingly broad within cortical areas, particularly retrosplenial (RSP), sensory areas (somatosensory [SS], and motor cortex [MO]), as well as the visual cortex (VIS) (Fig. 1b, red circle). Furthermore, to define the *Gpr88* signature on the mouse brain connectome, we ranked the brain areas on the basis of the number of statistically significant differences ($p < 0.01$, FDR corrected) in connectivity across the two genotypes (Fig. 1c). There was a clear dominance of connectivity changes for cortical-related nodes, with seven nodes from the top 10 being associated with isocortex. RSP showed the strongest remodeling (rank 1, Fig. 1c) of connectivity. Along with this area—classically included in the default mode network (DMN) across species (Raichle, 2015), VIS (rank 4, Fig. 1c), thalamus (TH, rank 5, Fig. 1c), temporal association area (TEa, rank 6, Fig. 1c), hippocampal formation (HPF, rank 8, Fig. 1c), and anterior cingulate area (ACA, rank 9, Fig. 1c) are brain regions present in the top 10 of our hierarchy. They were previously described as part of a DM-like network in the C57Bl/6 mouse strain (Liska et al., 2015; Hübner et al., 2017). This result suggests a strong modification of resting-state brain activity in *Gpr88*^{-/-} mice involving DMN.

Additional to this network, the intergroup comparison of FC matrices revealed significant changes (two-sample t -test, $p < 0.01$, FDR corrected) of the MO (rank 3, Fig. 1c) and the sensory (SS, rank 2; and VIS, rank 4, Fig. 1c) cortical connectivity, as well as the subcortical striatal (particularly involving the caudate-putamen [CP], rank 7, Fig. 1c) and HPF (rank 8, Fig. 1c) circuitries. Moreover, superior colliculus (SC) of the midbrain area—a major node for mediating sensorimotor transformations (Simon, 2008), was ranked tenth among the most altered brain regions (SC, rank 10, Fig. 1c). All together, these findings suggest several salient features of the *Gpr88*^{-/-} mouse brain architecture. These are massive intracortical and cortico-subcortical rsFC modifications, particularly involving DMN core areas along with the sensorimotor and cortico-striatal pathway. To further strengthen these findings, we performed seed analysis using the brain areas highlighted in the ICASSO-based ranking and anatomically defining the seeds on the basis of coregistration with the AMBA (see the Methods section).

*Altered DMN patterns in *Gpr88*^{-/-} mice*

The RSP was ranked on top of substantial FC differences across CTRL and *Gpr88*^{-/-} mice. We selected bilateral RSP as a seed region for mapping its connectivity patterns across the whole brain (two-tailed t -test, $p < 0.001$) and identify the DMN. Similar to previous work using the seed-based correlation approach (Hübner et al., 2017; Sforazzini et al., 2014), our results support the idea of a mouse DMN network with

RSP as a core area. Its positive connections with the medial and caudal ACA, HPF, TEa, and VIS system (Fig. 2a) portray, in the CTRL group, similarities with the posterior DMN obtained for humans (Di and Biswal, 2014). Relative to this CTRL pattern, RSP in the *Gpr88*^{-/-} group exhibited clearly reduced FC with ACA, TEa, and massive decreased FC with TH (Fig. 2a vs. b, c). This result demonstrates modification of the DMN in mice lacking the GPR88 receptor. This major modification, which we did not observe in mu opioid receptor knockout mice in our previous work (Mechling et al., 2016), is consistent with the large extent of behavioral alterations reported in *Gpr88*^{-/-} mice.

Remodeling of motor and sensory cortical rsFC in *Gpr88*^{-/-} mice

Sensory (SS) and motor (MO) areas were ranked second and third in the quantification of FC alterations of *Gpr88*^{-/-} mice. Seed-based analysis using MO as ROI revealed extensive cortical and subcortical rsFC modifications in the *Gpr88*^{-/-} group (Fig. 3a). We quantified the group statistical significance of the alterations using voxel-level general linear modeling corrected for multiple comparisons using the random field theory approach ($p < 0.001$, see the Materials and Methods section). When compared with the CTRL group, *Gpr88*^{-/-} MO showed salient features of reduced (Fig. 3b, CTRL > *Gpr88*^{-/-}) or stronger (Fig. 3b, CTRL < *Gpr88*^{-/-}) rsFC with specific brain areas.

From positive correlation analysis (Fig. 3a, correlations from 0 to 1, and Fig. 3b—positive correlation), the *Gpr88*^{-/-} group showed decreased (CTRL > *Gpr88*^{-/-}) FC between MO and orbitofrontal cortex, agranular insular area (AI), and

limbic areas (prelimbic cortex—PL, infralimbic cortex—ILA, nucleus accumbens—ACB), as well as caudal RSP and parietal cortex (PTLp). However, stronger rsFC (Fig. 3b, positive correlation CTRL < *Gpr88*^{-/-}) was quantified between MO and CP and MO and SS, as well as within the MO. These strong modifications of striato-motor connectivity are particularly relevant to highest *Gpr88* expression in the striatum and the hyperactive phenotype observed in this model (Logue et al., 2009; Meersman et al., 2016a; Quintana et al., 2012). **Negative correlation analysis** (Fig. 3a, correlations from -1 to 0, and Fig. 3b—anticorrelations) revealed reduced MO connectivity with amygdala (AMY) and posterior thalamic nuclei (TH) (Fig. 3b, anticorrelation CTRL > *Gpr88*^{-/-}), while stronger anticorrelations in the *Gpr88*^{-/-} group compared with CTRL were obtained with HPF and midbrain (MB) areas, including superior colliculus (SC) or periaqueductal gray (PAG).

Along with remodeled MO connectivity, the *Gpr88*^{-/-} SS cortex showed significant rsFC alterations (Fig. 3c, d). From positive correlation analysis (Fig. 3c, correlations from 0 to 1, and Fig. 3d—positive correlation), the *Gpr88*^{-/-} group revealed decreased rsFC (CTRL > *Gpr88*^{-/-}) between SS and the mid-caudal isocortex, including MO, RSP, and VIS areas. Stronger rsFC (Fig. 3d, positive correlation CTRL < *Gpr88*^{-/-}) was quantified between SS and rostral isocortex, including MO and ACA. These intracortical modifications of rsFC in the *Gpr88*^{-/-} group correlate with modifications of the brain connectivity matrix derived from ICASSO analysis (Fig. 1b). **Negative correlation analysis** (Fig. 3c, correlations from -1 to 0, and Fig. 3d—anticorrelations) revealed reduced SS connectivity with AMY, TH, hypothalamus (HY), and MB areas (Fig. 3d, anticorrelation CTRL > *Gpr88*^{-/-}), while

FIG. 2. *Gpr88* deletion strongly reshapes the DMN pattern, defined as positive RSP cortex FC: (a) DMN pattern in the CTRL animal group. RSP cortex demonstrated strong coherent fluctuations of the BOLD signal with rostromedial ACA, TEa, and VIS areas, HPF, and thalamus. (b) Altered DMN pattern in the *Gpr88*^{-/-} mouse brains is characterized by abolished FC of RSP with rostral ACA, TEa, and subcortical brain regions (HPF and TH), but strong connections mostly with the VIS area, including superior colliculus. (c) Statistically significant differences in the DMN patterns when comparing CTRL and *Gpr88*^{-/-} groups (GLM, $p < 0.001$, corrected). The left panel shows the brain regions positively correlated with RSP for which correlations are significantly stronger in the CTRL than in the *Gpr88*^{-/-} group. The right panel shows areas with FC stronger in the mutant group compared with the CTRL. The color scale at the middle indicates the corresponding *T*-value. BOLD, blood oxygen level-dependent; DMN, default mode network; GLM, general linear modeling.

FIG. 3. Remodeled sensory-motor FC underscores the hyperactive or distracted behavior in mutant mice: BOLD rsfMRI correlation maps for (a) MO and (c) SS. Corresponding correlation maps of the CTRL and *Gpr88*^{-/-} groups were overlaid on atlas brain slices (two-tailed t -test, $p < 0.001$). The color scale indicates the correlation value (positive correlations from 0 to +1: dark red to yellow and negative correlations from 0 to -1: dark blue to turquoise). (b, d) Comparative analysis of the statistically significant FC (GLM, $p < 0.001$, corrected) resulting from the seed regions: MO and SS, respectively. The left panel shows positive and anticorrelated brain regions with the respective seed, stronger in the CTRL than *Gpr88*^{-/-} group. The right panel shows more strongly correlated regions in the mutant group compared with the CTRL. The color scale at the middle indicates the corresponding T -value. rsfMRI, resting-state functional magnetic resonance imaging.

stronger anticorrelations in the *Gpr88*^{-/-} group were obtained between SS and ACB, HPF, and MB areas, including SC or PAG (Fig. 3d, anticorrelation CTRL < *Gpr88*^{-/-}).

Modified caudate-putamen and HPF connectivity in *Gpr88*^{-/-} mice

Other brain areas were ranked high in the quantification of FC alterations in mutant mice. Notable are caudate-

putamen (CP) and HPF, where *Gpr88* is highly enriched for the former and virtually absent for the latter. Our previous behavioral study investigated whether altered striatal function would translate into a modification of hippocampal/striatal balance in learning (Meirsman et al., 2016a). A dedicated, dual-solution cross-maze task revealed that *Gpr88*^{-/-} mice perform better in the allocentric versus egocentric component of the task for both acquisition and reversal learning, demonstrating facilitation of hippocampus-dependent behavior at the

expense of striatal-dependent responses (Meersman et al., 2016a). We therefore compared FC across these two brain structures using seed-based correlation analysis.

From *positive correlation analysis* (Fig. 4a, correlations from 0 to 1, and Fig. 4b—positive correlation), the *Gpr88*^{-/-} CP showed strongly decreased rsFC toward HPF, TH, and MB. CP rsFC was, however, increased in the *Gpr88*^{-/-} mice (Fig. 4b, positive correlation CTRL < *Gpr88*^{-/-}) toward ACA and rostral subcortical area, including septal

complex (S), pallidum (PAL), bed nuclei of the stria terminalis (BST), and AI. *Negative correlation analysis* (Fig. 4a, correlations from -1 to 0, and Fig. 4b—anticorrelations) revealed reduced CP connectivity with AMY, entorhinal (ENT), and VIS cortices, as well as SC and pontine olivary nuclei (P), in the *Gpr88*^{-/-} group. However, stronger anticorrelations were obtained in the *Gpr88*^{-/-} group between CP and ACB, as well as MO, SS, ACA, and PTLp cortical areas.

FIG. 4. FC patterns of dorsal striatum (CP) (a, b) and hippocampal formation (HPF) (c, d) are altered in *Gpr88*^{-/-} mouse brains: BOLD rsfMRI correlation maps for (a) CP and (c) HPF. Corresponding correlation maps of the CTRL and *Gpr88*^{-/-} groups were overlaid on T₂-weighted anatomical brain slices (two-tailed *t*-test, *p* < 0.001). The color scale indicates the correlation value (positive correlations from 0 to +1: dark red to yellow and negative correlations from 0 to -1: dark blue to turquoise). (b, d) Detailed analysis of the statistically significant FC modifications (GLM, *p* < 0.001, corrected) of CP and HPF in the *Gpr88*^{-/-} group. The left panel shows positive and anticorrelated brain regions with the respective seed stronger in the CTRL than *Gpr88*^{-/-} group, while the right panel shows more strongly correlated regions in the mutant group compared with the CTRL. The color scale at the middle indicates the corresponding *T*-value.

Seed-based correlation analysis quantified strong hippocampal connectivity alterations in the *Gpr88*^{-/-} group. *Positive correlation analysis* (Fig. 4c, correlations from 0 to 1, and Fig. 4d—positive correlation) showed decreased positive correlations between HPF and AMY, ENT, and TEa, as well as rostral TH and MB in the *Gpr88*^{-/-} group. Meanwhile, HPF increased its positive rsFC toward caudal TH nuclei, SC, rostral MB, and pontine gray (PG) (Fig. 4d—positive correlation CTRL < *Gpr88*^{-/-}). *Negative correlation analysis* (Fig. 4b, correlations from -1 to 0, and Fig. 4d—anticorrelations) of HPF network indicated decreased connectivity between HPF and frontal limbic system, including orbital (ORB), PL, and ACB areas, as well as ACA, MO, SS, RSP, and PTLp cortical regions, in the *Gpr88*^{-/-} mice. Increased anticorrelated rsFC was, however, quantified between HPF and CP, lateral septal nuclei (LSX), and ILA (Fig. 4d—anticorrelation CTRL < *Gpr88*^{-/-}). Altogether, the extensive striato-hippocampal rsFC modifications corroborate the modified striato-hippocampal learning phenotype that we previously described for *Gpr88*^{-/-} mice (Meersman et al., 2016a).

Structural connectivity assessment

To noninvasively verify if *Gpr88* gene deletion impacted the underlying microstructure of brain functional networks, we used DTI and fiber tractography-derived parameters (FD) for measuring structural connectivity modifications (Fig. 6). Significant increase of FD values was detected in *Gpr88*^{-/-} animals compared with the CTRL group (voxel-wise statistical group comparison, $p < 0.05$, FWE corrected, contrast CTRL < *Gpr88*^{-/-}) in brain areas with altered rsFC. These areas included CP, MO, SS, HPF, parts of TH, and MB. No significant changes could be detected when examining the CTRL > *Gpr88*^{-/-} for FD contrast. For generation of FD maps, we used a global fiber-tracking algorithm that was previously validated for mouse and human DTI data (see the Materials and Methods section). A similar pattern of significant modifications was also quantified when performing group statistics on the fractional anisotropy maps (data not shown) derived after calculation of the diffusion tensor.

Discussion

During rest, endogenous fluctuations in low-frequency BOLD signals are synchronized between different regions—widely distributed throughout the brain, forming dynamic FC networks (Cabral et al., 2014). The genetic and molecular factors that regulate the development and behavior of these networks remain undefined. In this study, we focused on the *Gpr88* gene and discovered significant modifications of brain connectional patterns in live *Gpr88*^{-/-} mice. This gene encodes an orphan G protein-coupled receptor whose ligand remains unknown and is expressed as early as embryonic day 16 in the rat (Massart et al., 2016) and P0 in the mouse (our unpublished data). Altered FC in mutant mice may therefore result either from the lack of GPR88 receptor activity during development, and thus reflect compensatory modifications, or from the absence of tonic GPR88 activity in the adult, or both. Inducible gene knockout approaches in the future should clarify respective contributions of early and adult GPR88 expression. The strong modifications of connectional patterns observed in adult *Gpr88*^{-/-}

mice may potentially underpin behavioral alterations of these mutant mice. Table 1 summarizes behavioral phenotypes reported for *Gpr88*^{-/-} mice, which relate to altered rsFC observed in this study, and the relevant connectivity alterations are summarized in Figure 5.

The repertoire of *Gpr88*^{-/-} mice behavioral phenotypes was often discussed with respect to dysfunctions of cell types and brain areas expressing *Gpr88* in normal conditions. Particularly, the aberrant activation of striatal GABAergic medium spiny neurons (Quintana et al., 2012)—in the absence of a functional GPR88 receptor—was suggested to be a major contributor to behavioral deficits observed in *Gpr88*^{-/-} mice. However, one has to consider that such perturbations of brain activity are not confined to a single locus, instead they spread along axonal pathways to influence other regions' activity and alter the way these areas communicate with each other. This rsfMRI study allowed a whole brain and hypothesis-free analysis of brain connectivity, unraveling the intrinsic mouse brain functional communication and highlighting the complex highly organized topology of functional networks as previously observed in rodents (Biswal et al., 1995; Liang et al., 2011; Ma et al., 2016; Smith et al., 2013; Zerbi et al., 2015; Zhang et al., 2010), with its central player, the DMN (Raichle et al., 2001). Defined as a set of brain regions that show high neuronal activity during rest (Fox and Raichle, 2007; Raichle, 2015), DMN raised a lot of interest as it was demonstrated that its coherent activity is perturbed in pathological conditions, including psychiatric disorders (Raichle, 2015; Zhou et al., 2014). We and other groups found the DM-like network in the healthy mouse brain (Grandjean et al., 2016; Hübner et al., 2017; Sforazzini et al., 2014) to be modified in mouse models of brain pathologies (Grandjean et al., 2016; Hübner et al., 2017). In this study, we show that *Gpr88* deletion in mice strongly perturbs the coherent activity of DMN, with major impact on the connectivity of RSP, the core player of this network. RSP connectivity with mid-rostral part of DMN, including the medial and anterior cingulate cortex as well as cortico-subcortical DMN subcomponents and temporal association area, is strongly suppressed in the *Gpr88*^{-/-} brains.

These large alterations of DMN upon deletion of the *Gpr88* gene are consistent with broad behavioral alterations mentioned in this mouse model and relate to patterns observed in human studies of psychiatric and neurologic disorders. Indeed FC modifications in the DMN have been reported, for instance, in bipolar disorder (Brady et al., 2016; Öngür et al., 2010; Wang et al., 2016), schizophrenia (Garritty et al., 2007; Kühn and Gallinat, 2012; Whitfield-Gabrieli and Ford, 2012), or attention-deficit/hyperactivity disorder (ADHD) (Castellanos and Proal, 2012; Castellanos et al., 2008; Fair et al., 2010; Fassbender et al., 2009; Hoekzema et al., 2014; Sonuga-Barke and Castellanos, 2007; Sun et al., 2012; Uddin et al., 2008). Emerging studies propose ADHD as a DMN disorder (Castellanos and Proal, 2012; Castellanos et al., 2008; Fair et al., 2010; Fassbender et al., 2009; Sonuga-Barke and Castellanos, 2007), and decreased DMN rsFC for adults (Castellanos et al., 2008; Hoekzema et al., 2014; Uddin et al., 2008) as well as adolescents suffering from ADHD (Sun et al., 2012) was reported. GPR88 receptor, as well as the behavioral traits of the *Gpr88*^{-/-} mice, was associated over time with the pathophysiology of such disorders (Del Zompo et al., 2014; Logue et al., 2009). This broad impact of *Gpr88* deletion on the

TABLE 1. SUMMARY BEHAVIORAL DEFICITS REPORTED IN *Gpr88*^{-/-} MICE

System	Behavioral test	Behavior results
Sensorimotor gating deficit	Prepulse inhibition of the acoustic startle response assay	Sensorimotor gating deficit (Logue et al., 2009)
Motor deficits and hyperactivity	Stereotypy	Increased stereotypy (Meirsman et al., 2016a)
	Rotarod	Impaired motor coordination or strength (Quintana et al., 2012)
	Grip test	No difference in muscle strength (Meirsman et al., 2016a)
	Basal locomotor activity	Increased basal locomotor activity in novel and familiar environments (Quintana et al., 2012)
Learning deficiency	Basal locomotor activity	Increased locomotor activity and lack of habituation to a novel environment. (Meirsman et al., 2016a)
	Basal locomotor activity	Increased locomotor activity (Meirsman et al., 2016b)
	Operant behavior, two-way active avoidance procedure	Impaired avoidance learning, acquisition, and integration of visual or auditory cues (Quintana et al., 2012)
	Morris water maze	Visuospatial memory and learning were intact (Quintana et al., 2012)
	A water-based U maze	Impairments in cue-based learning (Quintana et al., 2012)
	Rotarod	Motor coordination and learning impairment (Meirsman et al., 2016a)
	Y-maze	Increased exploration in new environments (Meirsman et al., 2016a)
	Novel object recognition test	Improved learning and recognition memory (Meirsman et al., 2016a)
	Dual-solution cross-maze task	Improved ability to distinguish between goal-directed responses and habitual behavior (Meirsman et al., 2016a)
	Fear conditioning	Impaired contextual fear and cue-related fear expression (Meirsman et al., 2016b)
Risk-taking behavior	Elevated-plus maze test	Reduced anxiety levels (Meirsman et al., 2016a)
	Elevated-plus maze test	Decreased anxiety behaviors (Meirsman et al., 2016b)
	Light-dark test	Exhibit increased risk-taking behaviors (Meirsman et al., 2016b)
	Marble-burying test	Lower anxiety behaviors (Meirsman et al., 2016a)
	Marble-burying test	Decreased threat avoidance and more risk-taking behaviors (Meirsman et al., 2016b)
	Nest building	Decreased anxiety (Meirsman et al., 2016a)
	Novelty preference	Increased novelty approach/low anxiety behaviors (Meirsman et al., 2016b)
	Novelty-suppressed feeding test	Decreased conflict anxiety (Meirsman et al., 2016a)
	Novelty-suppressed feeding test	Increased novelty approach/low anxiety behaviors (Meirsman et al., 2016b)
	Social interaction test	Increased social behaviors (Meirsman et al., 2016b)

Mutant mouse phenotypes are displayed in relation to brain connectivity domains as represented in Figure 5.

FIG. 5. Schematic representation of dominant resting-state FC alterations in *Gpr88*^{-/-} mice, associated with specific behavioral deficits previously reported for these mutant mice, and summarized in Table 1. Bold lines represent stronger correlations between regions, whereas dashed lines represent weaker correlations.

topology of resting-state connectivity has to be considered in a developmental context as well, as in normal brain, *Gpr88* shows differential expression over time in various brain areas. It is therefore likely that the global remodeling observed here results from the lack of GPR88 during development, at least for a large part.

Beside a strongly modified DMN pattern, we show extensive alterations of whole brain *Gpr88*^{-/-} FC matrix (Fig. 1) and, most interestingly, within intracortical connectivity. Indeed, recent work demonstrates intracellular redistribution of GPR88 during cortical lamination in the normal brains (Massart et al., 2016). In the cortical plate of the developing cortex, GPR88 presents a classical G protein-coupled receptor (GPCR) plasma membrane/cytoplasmic localization that shifts, on the day of birth, to nuclei of neurons progressively settling during postnatal development in layers V to II. It is likely that *Gpr88* influences, to some extent, the development of intracortical functional communication and that deletion of the receptor in *Gpr88*^{-/-} mice leads to remodeling of cortical functional pathways, as seen here. Particularly clear is the alteration of somatomotor connectivity (SS-MO) and SS-MO-ACA functional connections linked with the observed sensorimotor gating deficiency (Logue et al., 2009; Meersman et al., 2016a) and risk-taking behavior (Meersman et al., 2016b) of *Gpr88*^{-/-} mice. Sensorimotor gating is the process of screening or gating of the sensory and motor/cognitive information to enable uninterrupted processing of the most salient aspects of the external and internal environment (Butler et al., 1990). Sensorimotor gating deficiency reflects

central inhibitory functioning deficiency and underlies symptoms of ADHD (Holstein et al., 2013) in adults and schizophrenia patients (Braff and Geyer, 1990; Geyer et al., 2001). Prepulse inhibition is a well-validated operational measure of sensorimotor gating in humans and animals (Geyer et al., 2001) that is found to be disrupted in *Gpr88*^{-/-} mice (Logue et al., 2009). Beyond the widespread whole-brain functional disconnections detected in schizophrenic patients (Liang et al., 2006; Welsh et al., 2010), several studies report specific rsFC disruption in DMN and sensorimotor networks (Kaufmann et al., 2015; Tang et al., 2012) similar to our findings in mice lacking the GPR88 receptor. These connectivity features are suggestive of inhibitory control deficits, hyperactivity, and impulsivity symptoms, as seen in human ADHD (Choi et al., 2013; McLeod et al., 2014; Mostert et al., 2016; Tian et al., 2008). Most remarkably, the cortico-striatal connectivity (particularly MO–striatum FC) is strongly perturbed in *Gpr88*^{-/-} mice, congruent to the cardinal resting-state network perturbations observed in ADHD (Castellanos and Proal, 2012; Castellanos et al., 2006; Oldehinkel et al., 2016).

Striatal *Gpr88*^{-/-} FC changes are paralleled by structural modification of the striato-cortical pathways (Fig. 6), revealed in our study through DTI and high-resolution fiber mapping (Harsan et al., 2013). This modified striatocortical circuitry structure (especially the CP-MO altered component) is consistent with rsFC data and may underpin the hyperactive behavior described in the *Gpr88*^{-/-} model (Meersman et al., 2016a; Quintana et al., 2012). The involvement of *Gpr88* in shaping

FIG. 6. Significant alterations of the fiber density in *Gpr88*^{-/-} mice: Statistical significance was evaluated using a two-sample *t*-test ($p < 0.05$, FWE corrected). The panel shows the regions with higher fiber density in the mutant mice compared with the CTRL. Corresponding *T*-value scale is shown. FWE, familywise error.

striatal connections and therefore underlying hyperactive features is supported by the most abundant expression of the receptor in this brain area, including CP (Ghate et al., 2007; Mizushima et al., 2000) and ACB.

Our analysis also indicates that amygdala connectivity to somatosensory and motor cortical area as well as caudate-putamen is weakened in *Gpr88* knockout mice. This observation is consistent with the notion that amygdala might now drive increased risk-taking (RT) behavior in a potentially dangerous environment, leading to an apparent reduced anxiety in *Gpr88*^{-/-} mice (Meirsmann et al., 2016b). This phenotype was previously associated with altered gene transcription, dopamine levels, and neuronal morphology in amygdala (Alisch et al., 2014; Yokoyama et al., 2005) and consistent with the rsFC patterns reported in young adult RT behavior (Cox et al., 2010; DeWitt et al., 2014; Touroutoglou et al., 2014). In addition, hypersynchrony of the BOLD signal in the striatum of *Gpr88*^{-/-} mice, as observed in our study (Fig. 4a, b), has been reported earlier as a major contributor to adolescent RT behavior (Galvan, 2010).

The striatum, densely populated by GPR88 receptors in normal animals, showed perturbed functional cross talk not only with the hippocampus but also the prefrontal cortex, limbic area, and MB in *Gpr88*^{-/-} mice. Additionally, HPF rsFC was altered. These striatal and hippocampal FC perturbations might underlie the modified learning phenotype of *Gpr88*^{-/-} mice, observed in a behavioral test that specifically addresses the striatum-hippocampus balance in learning (Meirsmann et al., 2016a; Quintana et al., 2012). The dorsal striatum (CP), a major hub of the basal ganglia network, is involved in several functional domains, including learning, cognition, and motivation (Mestres-Missé et al., 2012; Miyachi et al., 2002; Yin et al., 2009). In rodents, CP lesions disrupt acquisition of habits and impair goal-directed learning (Yin et al., 2004, 2005). Human neuroimaging studies also report the involvement of CP activity in the development of habits and goal-directed behavior (Liljeholm et al., 2011; Tanaka et al., 2008; Tricomi et al., 2009). Hippocampus, on the other hand, plays crucial roles in working and episodic memory (Aggleton and Brown, 2006; Cabeza and Nyberg, 2000). Our study therefore reveals a particular deficit in the striatal-hippocampal dialog at the neural network level, which likely explains the preferred allocentric (hippocampus) versus egocentric (striatum) behavioral strategy adopted by mutant mice in performing the cross-maze task (Meirsmann et al., 2016a).

Thus far, the intrinsic functional architecture revealed spatially distinct brain regions that exhibit altered rsFC in the *Gpr88*^{-/-} mice, concordant with hyperactive characteristics of mutant mice and evocative of connectivity alterations observed in ADHD patients. In humans, disrupted functional communications between brain regions is often accompanied by microstructural abnormalities in the white matter, which are thought to contribute to behavioral functioning in ADHD (Nagel et al., 2011). Therefore, we also measured the microstructural integrity in the *Gpr88*^{-/-} mouse brain through DTI and fiber tractography (Harsan et al., 2013) and mapped the FD, one of the primary indices used to quantitatively measure structural integrity (Konrad and Eickhoff, 2010; Nagel et al., 2011), with diffusion-based tractography. FD values were significantly higher in *Gpr88*^{-/-} mice (two-sample *t*-test, $p < 0.05$, FWE corrected) particularly along the

striato-cortical pathway (Fig. 6) linking the striatum (CP) and cortical areas, like MO and SS. Former studies report disturbed structural connectivity of the cortico-striatal network in both adults and children with ADHD in comparison with healthy subjects (Konrad and Eickhoff, 2010; Tamm et al., 2012). Taken together, functional and structural connectivity modifications in the sensorimotor and cortico-striatal circuitry observed in *Gpr88*^{-/-} mice are consistent with the prevailing neurobiological hypothesis of ADHD, which identifies these networks as a probable substrate for cognitive and behavioral impairments seen in ADHD patients (Bush et al., 2005; Castellanos and Proal, 2012; Castellanos et al., 2006; Holstein et al., 2013; Oldehinkel et al., 2016; van Ewijk et al., 2012).

Conclusion

In conclusion, we provide here the first evidence of *Gpr88* involvement in reshaping the mouse brain functional and structural circuitry. The remodeled network architecture and topology underlie the large behavior deficits described in mice lacking a functional GPR88 receptor. Our study therefore suggests GPR88 as an influential player in brain connectivity and likely a susceptibility gene for psychiatric conditions in humans.

Acknowledgments

This project was funded with support from the NeuroTime Erasmus+: Erasmus Mundus program of the European Commission. This publication/communication reflects the views only of the author, and the Commission cannot be held responsible for any use that may be made of the information contained therein. This study was supported by the ATHOS Consortium (Fonds Unique Interministériel, Région Alsace, Domain Therapeutics Illkirch, France, and Prestwick Chemicals Illkirch, France). The authors also thank the National Institutes of Health (NIH-NIAAA No. 16658 and NIH-NIDA No. 005010) for financial support. The authors acknowledge the funding grants from Brain Links Brain Tools (BLBT) cluster of excellence from Freiburg (MouseNet 31 project).

Author Disclosure Statement

No competing financial interests exist.

References

- Aggleton JP, Brown MW. 2006. Interleaving brain systems for episodic and recognition memory. *Trends Cogn Sci* 10:455–463.
- Alisch RS, Chopra P, Fox AS, Chen K, White ATJ, Roseboom PH, et al. 2014. Differentially methylated plasticity genes in the amygdala of young primates are linked to anxious temperament, an at risk phenotype for anxiety and depressive disorders. *J Neurosci* 34:15548–15556.
- Becker JA, Befort K, Blad C, Filliol D, Ghate A, Dembele D, et al. 2008. Transcriptome analysis identifies genes with enriched expression in the mouse central extended amygdala. *Neuroscience* 156:950–965.
- Biswal B, Yetkin FZ, Haughton VM, Hyde JS. 1995. Functional connectivity in the motor cortex of resting human brain using echo-planar MRI. *Magn Reson Med* 34:537–541.
- Brady RO, Tandon N, Masters GA, Margolis A, Cohen BM, Keshavan M, Öngür D. 2016. Differential brain network

- activity across mood states in bipolar disorder. *J Affect Disord* 207:367–376.
- Braff DL, Geyer MA. 1990. Sensorimotor gating and schizophrenia. Human and animal model studies. *Arch Gen Psychiatry* 47:181–188.
- Bush G, Valera EM, Seidman LJ. 2005. Functional neuroimaging of attention-deficit/hyperactivity disorder: a review and suggested future directions. *Biol Psychiatry* 57:1273–1284.
- Butler RW, Braff DL, Rausch JL, Jenkins MA, Sprock J, Geyer MA. 1990. Physiological evidence of exaggerated startle response in a subgroup of Vietnam veterans with combat-related PTSD. *Am J Psychiatry* 147:1308–1312.
- Cabeza R, Nyberg L. 2000. Imaging cognition II: an empirical review of 275 PET and fMRI studies. *J Cogn Neurosci* 12:1–47.
- Cabral J, Kringelbach ML, Deco G. 2014. Exploring the network dynamics underlying brain activity during rest. *Prog Neurobiol* 114:102–131.
- Calhoun VD, Adali T, Pearlson GD, Pekar JJ. 2001. A method for making group inferences from functional MRI data using independent component analysis. *Hum Brain Mapp* 14:140–151.
- Castellanos FX, Margulies DS, Kelly C, Uddin LQ, Ghaffari M, Kirsch A, et al. 2008. Cingulate-precuneus interactions: a new locus of dysfunction in adult attention-deficit/hyperactivity disorder. *Biol Psychiatry* 63:332–337.
- Castellanos FX, Proal E. 2012. Large-scale brain systems in ADHD: beyond the prefrontal-striatal model. *Trends Cogn Sci* 16:17–26.
- Castellanos FX, Sonuga-Barke EJS, Milham MP, Tannock R. 2006. Characterizing cognition in ADHD: beyond executive dysfunction. *Trends Cogn Sci* 10:117–123.
- Choi J, Jeong B, Lee SW, Go HJ. 2013. Aberrant development of functional connectivity among resting state-related functional networks in medication-naïve ADHD children. *PLoS One* 8:e83516.
- Cox CL, Gotimer K, Roy AK, Castellanos FX, Milham MP, Kelly C. 2010. Your resting brain CAREs about your risky behavior. *PLoS One* 5:e12296.
- Del Zompo M, Deleuze JF, Chillotti C, Cousin E, Niehaus D, Ebstein RP, et al. 2014. Association study in three different populations between the GPR88 gene and major psychoses. *Mol Genet Genomic Med* 2:152–159.
- DeWitt SJ, Aslan S, Filbey FM. 2014. Adolescent risk-taking and resting state functional connectivity. *Psychiatry Res Neuroimaging* 222:157–164.
- Di X, Biswal BB. 2014. Modulatory interactions between the default mode network and task positive networks in resting-state. *PeerJ* 2:e367.
- Esposito F, Seifritz E, Formisano E, Morrone R, Scarabino T, Tedeschi G, Cirillo S, Goebel R, Di Salle F. 2003. Real-time independent component analysis of fMRI time-series. *Neuroimage* 20:2209–2224.
- Fair DA, Posner J, Nagel BJ, Bathula D, Dias TGC, Mills KL, et al. 2010. Atypical default network connectivity in youth with attention-deficit/hyperactivity disorder. *Biol Psychiatry* 68:1084–1091.
- Fassbender C, Zhang H, Buzy WM, Cortes CR, Mizuiri D, Beckett L, Schweitzer JB. 2009. A lack of default network suppression is linked to increased distractibility in ADHD. *Brain Res* 1273:114–128.
- Fox MD, Raichle ME. 2007. Spontaneous fluctuations in brain activity observed with functional magnetic resonance imaging. *Nat Rev Neurosci* 8:700–711.
- Galvan A. 2010. Adolescent development of the reward system. *Front Hum Neurosci* 4:6.
- Garrity AG, Pearlson GD, McKiernan K, Lloyd D, Kiehl KA, Calhoun VD. 2007. Aberrant “default mode” functional connectivity in schizophrenia. *Am J Psychiatry* 164:450–457.
- Geyer MA, Krebs-Thomson K, Braff DL, Swerdlow NR. 2001. Pharmacological studies of prepulse inhibition models of sensorimotor gating deficits in schizophrenia: a decade in review. *Psychopharmacology (Berl.)* 156:117–154.
- Ghate A, Befort K, Becker JAJ, Filliol D, Bole-Feysot C, Demebele D, et al. 2007. Identification of novel striatal genes by expression profiling in adult mouse brain. *Neuroscience* 146:1182–1192.
- Grandjean J, Azzinnari D, Seuwen A, Sigrist H, Seifritz E, Pryce CR, Rudin M. 2016. Chronic psychosocial stress in mice leads to changes in brain functional connectivity and metabolite levels comparable to human depression. *Neuroimage* 142:544–552.
- Harsan LA, Dávid C, Reiser M, Schnell S, Hennig J, von Elverfeldt D, Staiger JF. 2013. Mapping remodeling of thalamocortical projections in the living reeler mouse brain by diffusion tractography. *Proc Natl Acad Sci U S A* 110: E1797–E1806.
- Himberg J, Hyvärinen A, Esposito F. 2004. Validating the independent components of neuroimaging time series via clustering and visualization. *Neuroimage* 22:1214–1222.
- Hoekzema E, Carmona S, Ramos-Quiroga JA, Richarte Fernández V, Bosch R, Soliva JC, et al. 2014. An independent components and functional connectivity analysis of resting state fMRI data points to neural network dysregulation in adult ADHD. *Hum Brain Mapp* 35:1261–1272.
- Holstein DH, Vollenweider FX, Geyer MA, Csomor PA, Belser N, Eich D. 2013. Sensory and sensorimotor gating in adult attention-deficit/hyperactivity disorder (ADHD). *Psychiatry Res* 205:117–126.
- Hübner N, Mechling AE, Lee HL, Reiser M, Bienert T, Hennig J, et al. 2017. The connectomics of brain demyelination: functional and structural patterns in the cuprizone mouse model. *Neuroimage* 146:1–18.
- Hyvärinen A, Oja E. 2000. Independent component analysis: algorithms and applications. *Neural Netw* 13:411–430.
- Kaufmann T, Skåtun KC, Alnæs D, Doan NT, Duff EP, Tønnesen S, et al. 2015. Disintegration of sensorimotor brain networks in schizophrenia. *Schizophr Bull* 41:1326–1335.
- Konrad K, Eickhoff SB. 2010. Is the ADHD brain wired differently? A review on structural and functional connectivity in attention deficit hyperactivity disorder. *Hum Brain Mapp* 31:904–916.
- Kühn S, Gallinat J. 2012. The neural correlates of subjective pleasantness. *Neuroimage* 61:289–294.
- Lein ES, Hawrylycz MJ, Ao N, Ayres M, Bensinger A, Bernard A, et al. 2007. Genome-wide atlas of gene expression in the adult mouse brain. *Nature* 445:168–176.
- Li Y-O, Adali T, Calhoun VD. 2007. Estimating the number of independent components for functional magnetic resonance imaging data. *Hum Brain Mapp* 28:1251–1266.
- Liang M, Zhou Y, Jiang T, Liu Z, Tian L, Liu H, Hao Y. 2006. Widespread functional disconnectivity in schizophrenia with resting-state functional magnetic resonance imaging. *Neuroreport* 17:209–213.
- Liang Z, King J, Zhang N. 2011. Uncovering intrinsic connective architecture of functional networks in awake rat brain. *J Neurosci* 31:3776–3783.

- Liljeholm M, Tricomi E, O'Doherty JP, Balleine BW. 2011. Neural correlates of instrumental contingency learning: differential effects of action-reward conjunction and disjunction. *J Neurosci* 31:2474–2480.
- Liska A, Galbusera A, Schwarz AJ, Gozzi A. 2015. Functional connectivity hubs of the mouse brain. *Neuroimage* 115: 281–291.
- Logue SF, Grauer SM, Paulsen J, Graf R, Taylor N, Sung MA, et al. 2009. The orphan GPCR, GPR88, modulates function of the striatal dopamine system: a possible therapeutic target for psychiatric disorders? *Mol Cell Neurosci* 42:438–447.
- Ma Z, Perez P, Ma Z, Liu Y, Hamilton C, Liang Z, Zhang N. 2016. Functional atlas of the awake rat brain: a neuroimaging study of rat brain specialization and integration. *Neuroimage*. pii: S1053-8119(16)30315-9.
- Margulies DS, Böttger J, Long X, Lv Y, Kelly C, Schäfer A, Goldhahn D, Abbushi A, Milham MP, Lohmann G, Villringer A. 2010. Resting developments: a review of fMRI post-processing methodologies for spontaneous brain activity. *MAGMA* 23:289–307.
- Massart R, Mignon V, Stanic J, Munoz-Tello P, Becker JAJ, Kieffer BL, et al. 2016. Developmental and adult expression patterns of the G-protein-coupled receptor GPR88 in the rat: establishment of a dual nuclear-cytoplasmic localization. *J Comp Neurol* 524:2776–2802.
- McLeod KR, Langevin LM, Goodyear BG, Dewey D. 2014. Functional connectivity of neural motor networks is disrupted in children with developmental coordination disorder and attention-deficit/hyperactivity disorder. *Neuroimage Clin* 4:566–575.
- Mechling AE, Arefin T, Lee HL, Bienert T, Reiser M, Ben Hamida S, et al. 2016. Deletion of the mu opioid receptor gene in mice reshapes the reward-aversion connectome. *Proc Natl Acad Sci U S A* 113:11603–11608.
- Mechling AE, Hübner NS, Lee HL, Hennig J, von Elverfeldt D, Harsan LA. 2014. Fine-grained mapping of mouse brain functional connectivity with resting-state fMRI. *Neuroimage* 96:203–215.
- Meirsmann AC, Le Merrer J, Pellissier LP, Diaz J, Clesse D, Kieffer BL, Becker JAJ. 2016a. Mice lacking GPR88 show motor deficit, improved spatial learning, and low anxiety reversed by delta opioid antagonist. *Biol Psychiatry* 79:917–927.
- Meirsmann AC, Robé A, de Kerchove d'Exaerde A, Kieffer BL. 2016b. GPR88 in A2AR neurons enhances anxiety-like behaviors. *eNeuro* 3. DOI:10.1523/ENEURO.0202-16.2016
- Mestres-Missé A, Turner R, Friederici AD. 2012. An anterior-posterior gradient of cognitive control within the dorsomedial striatum. *Neuroimage* 62:41–47.
- Miyachi S, Hikosaka O, Lu X. 2002. Differential activation of monkey striatal neurons in the early and late stages of procedural learning. *Exp Brain Res* 146:122–126.
- Mizushima K, Miyamoto Y, Tsukahara F, Hirai M, Sakaki Y, Ito T. 2000. A novel G-protein-coupled receptor gene expressed in striatum. *Genomics* 69:314–321.
- Moritz CH, Carew JD, McMillan AB, Meyerand ME. 2005. Independent component analysis applied to self-paced functional MR imaging paradigms. *Neuroimage* 25:181–192.
- Mostert JC, Shumskaya E, Mennes M, Onnink AMH, Hoogman M, Kan CC, et al. 2016. Characterising resting-state functional connectivity in a large sample of adults with ADHD. *Prog Neuropsychopharmacol Biol Psychiatry* 67:82–91.
- Nagel BJ, Bathula D, Herting M, Schmitt C, Kroenke CD, Fair D, Nigg JT. 2011. Altered white matter microstructure in children with attention-deficit/hyperactivity disorder. *J Am Acad Child Adolesc Psychiatry* 50:283–292.
- Oldehinkel M, Beckmann CF, Pruim RHR, van Oort ESB, Franke B, Hartman CA, et al. 2016. Attention-deficit/hyperactivity disorder symptoms coincide with altered striatal connectivity. *Biol Psychiatry Cogn Neurosci Neuroimaging* 1:353–363.
- Öngür D, Lundy M, Greenhouse I, Shinn AK, Menon V, Cohen BM, Renshaw PF. 2010. Default mode network abnormalities in bipolar disorder and schizophrenia. *Psychiatry Res* 183:59–68.
- Quintana A, Sanz E, Wang W, Storey GP, Güler AD, Wanat MJ, et al. 2012. Lack of GPR88 enhances medium spiny neuron activity and alters motor- and cue-dependent behaviors. *Nat Neurosci* 15:1547–1555.
- Raichle ME. 2015. The brain's default mode network. *Annu Rev Neurosci* 38:433–447.
- Raichle ME, MacLeod AM, Snyder AZ, Powers WJ, Gusnard DA, Shulman GL. 2001. A default mode of brain function. *Proc Natl Acad Sci U S A* 98:676–682.
- Reiser M, Mader I, Anastasopoulos C, Weigel M, Schnell S, Kiselev V. 2011. Global fiber reconstruction becomes practical. *Neuroimage* 54:955–962.
- Richiardi J, Altmann A, Milazzo AC, Chang C, Chakravarty MM, Banaschewski T, et al. 2015. Correlated gene expression supports synchronous activity in brain networks. *Science* 348:1241–1244.
- Sawiak SJ, Wood NI, Williams GB, Morton AJ, Carpenter TA. 2013. Voxel-based morphometry with templates and validation in a mouse model of Huntington's disease. *Magn Reson Imaging* 31:1522–1531.
- Sforazzini F, Schwarz AJ, Galbusera A, Bifone A, Gozzi A. 2014. Distributed BOLD and CBV-weighted resting-state networks in the mouse brain. *Neuroimage* 87:403–415.
- Simon SS. 2008. Merging of the senses. *Front Neurosci* 2:13–14.
- Smith SM, Beckmann CF, Andersson J, Auerbach EJ, Bijsterbosch J, Douaud G, et al. 2013. Resting-state fMRI in the Human Connectome Project. *Neuroimage* 80:144–168.
- Sonuga-Barke EJS, Castellanos FX. 2007. Spontaneous attentional fluctuations in impaired states and pathological conditions: a neurobiological hypothesis. *Neurosci Biobehav Rev* 31:977–986.
- Sun L, Cao Q, Long X, Sui M, Cao X, Zhu C, et al. 2012. Abnormal functional connectivity between the anterior cingulate and the default mode network in drug-naïve boys with attention deficit hyperactivity disorder. *Psychiatry Res* 201:120–127.
- Tamm L, Barnea-Goraly N, Reiss AL. 2012. Diffusion tensor imaging reveals white matter abnormalities in Attention-Deficit/Hyperactivity Disorder. *Psychiatry Res* 202:150–154.
- Tanaka SC, Balleine BW, O'Doherty JP. 2008. Calculating consequences: brain systems that encode the causal effects of actions. *J Neurosci* 28:6750–6755.
- Tang Y, Wang L, Cao F, Tan L. 2012. Identify schizophrenia using resting-state functional connectivity: an exploratory research and analysis. *Biomed Eng Online* 11:50.
- Thompson PM, Ge T, Glahn DC, Jahanshad N, Nichols TE. 2013. Genetics of the connectome. *Neuroimage* 80:475–488.
- Tian L, Jiang T, Liang M, Zang Y, He Y, Sui M, Wang Y. 2008. Enhanced resting-state brain activities in ADHD patients: a fMRI study. *Brain Dev* 30:342–348.
- Touroutoglou A, Bickart KC, Barrett LF, Dickerson BC. 2014. Amygdala task-evoked activity and task-free connectivity independently contribute to feelings of arousal. *Hum Brain Mapp* 35:5316–5327.
- Uddin LQ, Kelly AMC, Biswal BB, Margulies DS, Shehzad Z, Shaw D, et al. 2008. Network homogeneity reveals decreased

- integrity of default-mode network in ADHD. *J Neurosci Methods* 169:249–254.
- van de Ven VG, Formisano E, Prvulovic D, Roeder CH, Linden DEJ. 2004. Functional connectivity as revealed by spatial independent component analysis of fMRI measurements during rest. *Hum Brain Mapp* 22:165–178.
- Van Essen DC. 2013. Cartography and connectomes. *Neuron* 80:775–790.
- van Ewijk H, Heslenfeld DJ, Zwiers MP, Buitelaar JK, Oosterlaan J. 2012. Diffusion tensor imaging in attention deficit/hyperactivity disorder: a systematic review and meta-analysis. *Neurosci Biobehav Rev* 36:1093–1106.
- Van Waes V, Tseng KY, Steiner H. 2011. GPR88- a putative signaling molecule predominantly expressed in the striatum: cellular localization and developmental regulation. *Basal Ganglia* 1:83–89.
- Wang Y, Zhong S, Jia Y, Sun Y, Wang B, Liu T, et al. 2016. Disrupted resting-state functional connectivity in nonmedicated bipolar disorder. *Radiology* 280:529–536.
- Welsh RC, Chen AC, Taylor SF. 2010. Low-frequency BOLD fluctuations demonstrate altered thalamocortical connectivity in schizophrenia. *Schizophr Bull* 36:713–722.
- Whitfield-Gabrieli S, Ford JM. 2012. Default mode network activity and connectivity in psychopathology. *Annu Rev Clin Psychol* 8:49–76.
- Worsley KJ, Marrett S, Neelin P, Vandal AC, Friston KJ, Evans AC. 1996. A unified statistical approach for determining significant signals in images of cerebral activation. *Hum Brain Mapp* 4:58–73.
- Yin HH, Knowlton BJ, Balleine BW. 2004. Lesions of dorsolateral striatum preserve outcome expectancy but disrupt habit formation in instrumental learning. *Eur J Neurosci* 19:181–189.
- Yin HH, Mulcare SP, Hilário MRF, Clouse E, Holloway T, Davis MI, et al. 2009. Dynamic reorganization of striatal circuits during the acquisition and consolidation of a skill. *Nat Neurosci* 12:333–341.
- Yin HH, Ostlund SB, Knowlton BJ, Balleine BW. 2005. The role of the dorsomedial striatum in instrumental conditioning. *Eur J Neurosci* 22:513–523.
- Yokoyama M, Suzuki E, Sato T, Maruta S, Watanabe S, Miyaoka H. 2005. Amygdalic levels of dopamine and serotonin rise upon exposure to conditioned fear stress without elevation of glutamate. *Neurosci Lett* 379:37–41.
- Zerbi V, Grandjean J, Rudin M, Wenderoth N. 2015. Mapping the mouse brain with rs-fMRI: an optimized pipeline for functional network identification. *Neuroimage* 123:11–21.
- Zhang N, Rane P, Huang W, Liang Z, Kennedy D, Frazier JA, King J. 2010. Mapping resting-state brain networks in conscious animals. *J Neurosci Methods* 189:186–196.
- Zhou F, Zhuang Y, Gong H, Wang B, Wang X, Chen Q, et al. 2014. Altered inter-subregion connectivity of the default mode network in relapsing remitting multiple sclerosis: a functional and structural connectivity study. *PLoS One* 9:e101198.

Address correspondence to:

Laura-Adela Harsan
Engineering Science, Computer Science
and Imaging Laboratory (ICube)
Integrative Multimodal Imaging in Healthcare
UMR 7357/University of Strasbourg—CNRS
4 Rue Kirschleger
Strasbourg 67000
France

E-mail: harsan@unistra.fr