MU-OPIOID RECEPTOR RELATED CHANGES IN THE MOUSE BRAIN CONNECTOME MAPPED VIA RESTING-STATE FUNCTIONAL AND DIFFUSION WEIGHTED MRI

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Introduction: Detecting spontaneous low frequency fluctuations in the BOLD signal and their temporal correlations, resting-state fMRI (rsfMRI) provides an indirect mean for investigating whole brain neuronal activity at rest. Recently, there is growing interest in translation of rsfMRI experiments in small animals, as a large number of human neurological disorders are modeled in mice and rats. **The main purpose** of our study was to map functional connectivity changes via rsfMRI in mu opioid receptor (MOR) knock-out mice (Oprm1$^{-/-}$) and to compare the findings with complementary diffusion based fiber tractography. The MOR is involved in the action of analgesic opiate drugs and the regulation of pain experience. Mouse resting-state (rs) datasets were probed using high dimensional spatial group independent component analysis (ICA) in combination with partial correlation and graph theory to finding reliable functional connectivity networks throughout the mouse brain. Our characterization of alterations caused by genetic inactivation of MOR **will be interesting** to both researchers and clinicians proposing non-invasive markers and potential targets for therapeutic pain therapy in mouse models as well as in patients.

Material and Methods: RsfMRI was performed in 12 weeks old male Oprm1$^{-/-}$ mice (n=14) and respective wild types (WT, n=14)[1] under medetomidine sedation (sc bolus 0.3 mg/kgBW, continuous sc infusion 0.6mg/kgBW, 200µl/h)[2]. Physiological parameters were monitored and stabilized during scanning performed with a 7T animal scanner and a mouse head adapted cryocoil (Biospec 70/20 and MRI CryoProbe, Bruker, Germany). RsfMRI data was acquired using single shot GE EPI (TE/TR = 10ms/1700ms) using 12 axial slices à 0.7mm, FOV 1.92x1.2cm², planar spatial resolution 150x150µm². 200 volumes were recorded for each run. Preprocessing was done using SPM8 for motion correction, spatial normalization and alignment with a study based template as well as smoothing (0.4x0.4x1mm³). Diffusion weighted data was acquired using four-short DT-EPI with 25 axial slices of 0.5mm, 100x100x500µm³ (TE/TR = 20ms/7750ms), Δ = 10ms, δ=4ms, b=1000s/mm², 30 non-collinear gradient diffusion directions. Group spatial Independent Component Analysis (via Group ICA of fMRI Toolbox, v1.3i, Calhoun et al.), partial correlation (PC) analysis of IC time courses and graph theory were combined[2] to create a comprehensive picture of the global architecture of the rs functional connectivity in WT and Oprm1$^{-/-}$ mice. 100 ICA was carried out on combined 28 rsfMRI data sets using ICASSO to evaluate the compactness and reliability of each identified component. 14 components were excluded covering cerebrovascular areas or being associated with movement. The remaining 86 IC time courses were then assessed using PC analysis (normalized using Fisher's Z transformation) and the positive correlations were the input for graph theory in order to reveal connector hubs for each experimental group (based on over-average strength and diversity)[3]. Global network topological metrics (global clustering coefficient, mean shortest path length, small-worldness, and modularity)[4] were also evaluated. The adjacency PC matrices including as well negative correlations were entered into a two-sample t-test of WT vs Oprm1$^{-/-}$ performed on each element in the Z matrices in order to probe differences in direct connectivity (p<0.05, FDR correction). Fiber tracking and generation of super-resolution fiber density maps was done using adapted global organization algorithm[5]. The functional clusters identified via ICA were subsequently used as ROIs for extraction of numbers of fibers as well as fractional anisotropy, radial/axial diffusivity connecting each pair of regions for comparable measures in underlying structural connectivity and its changes.

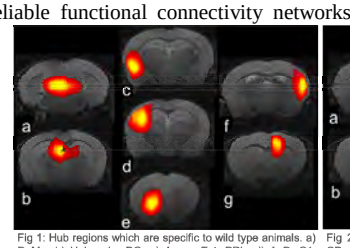


Fig 1: Hub regions which are specific to wild type animals. a) DpMe; b) Habenula, DG; c) Amyg, Ect, PRh; d) AuD, S1, V2L, CA2+3; e) CPu, NAc; f) S1BF, S2, GI, DI; g) M2, Cg1+2

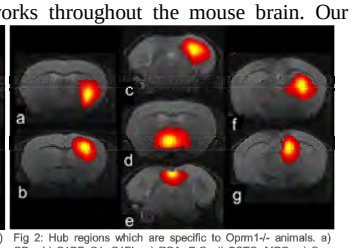


Fig 2: Hub regions which are specific to Oprm1-/- animals. a) CPu; b) S1BF, S1, S1FL; c) RSA, PrS; d) BSTS, MPO; e) Sup Col; f) DLG, LPM, IMA, LPLR, Po; g) RSG

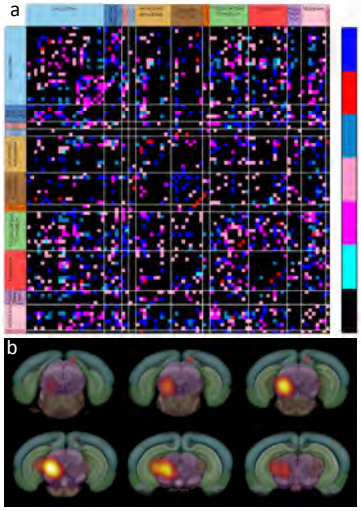
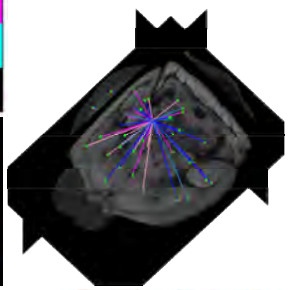


Fig 3: a) Changes in connectivity (p<0.05, FDR corrected, WT (c1) vs Oprm1$^{-/-}$(c2)), rearrangement of ICA components according to broader brain areas. Legend

No significant change:	black
c1-c2 = 1, c1&c2<0:	lightblue
c1-c2 = 1, c1&c2>0:	pink
c1-c2 = 1, c1>0, c2<0:	rosé
c1-c2 = -1, c1&c2<0:	blue-gray
c1-c2 = -1, c1&c2>0:	red
c1-c2 = -1, c1<0, c2>0:	blue

b) Thalamus: component with highest number of changes overlaid on Allen Brain Atlas based template and c) respective altered connections.



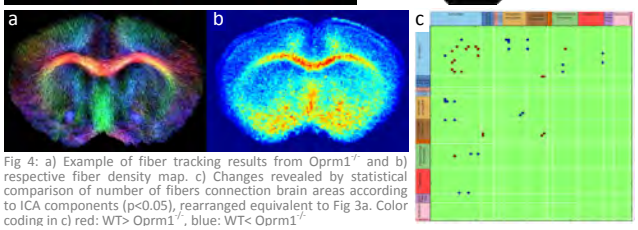


Fig 4: a) Example of fiber tracking results from Oprm1$^{-/-}$ and b) respective fiber density map. c) Changes revealed by statistical comparison of number of fibers connection brain areas according to ICA components (p<0.05), rearranged equivalent to Fig 3a. Color coding in c) red: WT> Oprm1$^{-/-}$, blue: WT< Oprm1$^{-/-}$

Results and Discussion: The rsfMRI data analysis revealed similar global topological features of MOR deprived vs WT mouse brains with an identical number of stable functional modules (6) as well as conserved small-worldness[4]. The importance of individual mouse brain regions in terms of their intra- and inter-modular connectivity was further assessed in both groups based on the normalized strength and diversity of each IC by using the weighted partial correlation results matrices[3]. The resulting ranking of the regions according to these two measures revealed hubs specific to Oprm1$^{-/-}$ (Fig 2) and missing in Oprm1$^{-/-}$ (Fig 1), the latter remarkably comprising two areas of highest mu opioid receptor density[6] – Habenula (Hb, Fig 1b) and Nucleus Accumbens (NAc, Fig 1e). This suggests a decreased importance of these regions due to the missing MOR. The group comparison based on both positive and negative correlations (Fig 3a) revealed an average number of altered functional connections of 18.7/IC. The IC with highest number of significant connectivity changes (37) was covering midbrain reticular nucleus, thalamic nuclei and periaqueductal gray (PAG, Fig 3b and c). Its altered functional connectivity include areas such as somatosensory cortex (SSC), prefrontal and limbic areas (PFC), extended amygdala, especially NAc and PAG – all brain regions known to participate in the circuitry of pain[7]. Additionally, one component – assigned to the spatial location of Hb, also shows extensive connectivity changes towards areas involved in the pain circuitry. Interestingly, many of the brain regions previously mentioned including PFC, NAc and Hb are also known to be important players in the reward circuitry[8]. Therefore our rsfMRI data strongly highlights this double player role and suggest an involvement of MOR in the reward-aversion circuitry. Much sparser changes of the structural connectivity were observed after fiber tracking (Fig 4). However, evidence for pain and reward circuitry remodeling are suggested by modifications in the fiber density in key areas of both circuitries. The wiring within NAc is decreased, as well as the one in between SSC and Amygdala in Oprm1$^{-/-}$ involved via indirect pathways in pain processing. Intriguing increased number of fibers connecting ventral striatum and prefrontal/limbic areas, regions known to be directly connected via dopaminergic neurons through the medial forebrain bundle was assessed. Lesions of the medial forebrain bundle are known to reduce rewarding effects of drugs of abuse like opiates and thus relate to the genetic modification induced here.

Conclusion: Spatial ICA, PC and Graph Theory allowed for discovery of large scale functional brain networks of similar global topology in Oprm1$^{-/-}$ and respective WT mice. Remodeling features were shown by the identification of Oprm1$^{-/-}$ specific hub regions, demonstrating the importance of reward system pathways. A statistical comparison of both groups revealed changes in the pain circuitry. Thus, our study on innate MOR deprived brain highlights a developmental process of functional and structural connectivity remodeling. The non-invasive methodological design used here offers the possibility of longitudinal investigations with individual follow-up of brain network fluctuations at different stages after inactivation of MOR and allows for evaluating possible new therapeutic compounds and their influence on the progress of disease patterns involving MOR such as addiction.

References: [1]Matthes et al, 1996, Nature; [2]Mechling et al, 2014, NeuroImage; [3]Rubinov&Sporns, 2011, NeuroImage; [4]Watts&Strogatz, 1998, Nature; [5]Harsan et al, 2013, PNAS; [6]Lutz and Kieffer, 2013, Trends Neurosci; [7]Bushnell et al, 2013, Nature RevNeurosci; [8]Russo&Nestler, 2013, Nature RevNeurosci