Mapping the mouse brain functional connectivity networks: strain specific patterns

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Introduction: Enormous effort has been focused during the last decade on addressing non-invasively the issue of intrinsic organization of brain functional connectivity (FC)[1]. Using resting state fMRI (rsfMRI), the FC architecture of the human brain and its dynamics has been consistently revealed in multiple networks that could be altered or remodeled by various normal physiological or pathological conditions. However, the intrinsic connectional architecture of functional networks (FN) in the mouse brain remains a significantly underexplored research area. The primary goal of our study was to bridge this gap, by systematically and comparatively probing the intrinsic brain FC of two mouse strains, intensively used in the fundamental and preclinical neuroscience : the C57Bl6/N and the Balbc/J strains. Particularly motivating was the investigation of topological organization of FN in a population of Balbc/J mice, a strain previously recognized for its great inter-individual variability in the organization of structural connectivity profiles[2, 3] and for its behavioral phenotypes resembling autism disorders[3]. Uncovering the large scale FC pattern in such models, in a strain specific manner, represents a first step towards a better understanding of modifications in basal, healthy state networks under theimpact of various factors, related with genetic, pharmacological or pathological conditions.

Materials and Methods:

RsfMRI was conducted in 8-9 weeks old C57BL/6N (n=13) and Balbc/J female mice (n=10). Animal anesthesia was initiated by a subcutaneous (sc) bolus injection of medetomidine (MD- Domitor,Pfizer, Germany) at a dose of 0.3mg MD per kg bw in 100 µl 0.9% NaCl-solution. A slight sedation was maintained throughout the imaging sessions by continuous sc infusion of MD (0.6mg per kg bw, 200µl/h). The physiological conditions (body temperature, respiration/heart rate, blood oxygen saturation) were monitored. All experiments were performed using a 7T / 20cm Biospec small bore animal scanner and a cryogenically cooled quadrature mouse brain resonator (Bruker, Germany). Data was acquired with a T_2*-weighted single shot Gradient Echo EPI (TE/TR = 10ms/1700ms, 12 axial slices of 0.7 mm thickness; 19x12 mm^2 FOV; in plane resolution of 150x150 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 a reference scan and smoothing (0.4x0.4x1mm^3). Group spatial Independent Component Analysis (ICA) using the MATLAB tool GIFT (Group ICA of fMRI Toolbox, v1.3i) was carried out on combined rsfMRI data sets using ICASSO (with 20 runs) to evaluate the reliability[4] of each identified component (network). The nr of components was set at 40 and the spatial maps of the independent components (IC) were scaled to z scores.

Partial correlation and graph theory: the time courses of the obtained IC were further used in partial correlation (PC) analysis. Two PC matrices were generated, corresponding to each group of animals. The ICs were considered as nodes and the correlation coefficient between pairs of components was assessed (weight). Focusing on positive correlations only, the PC matrices were converted into binary ones using a $p<0.05$ relevance threshold and the brain regions (IC or nodes) with the highest number of statistically relevant connections were identified. The strength of each node was also calculated (average weight of relevant positive connections) and finally functional modules were identified using the graph theory[5].

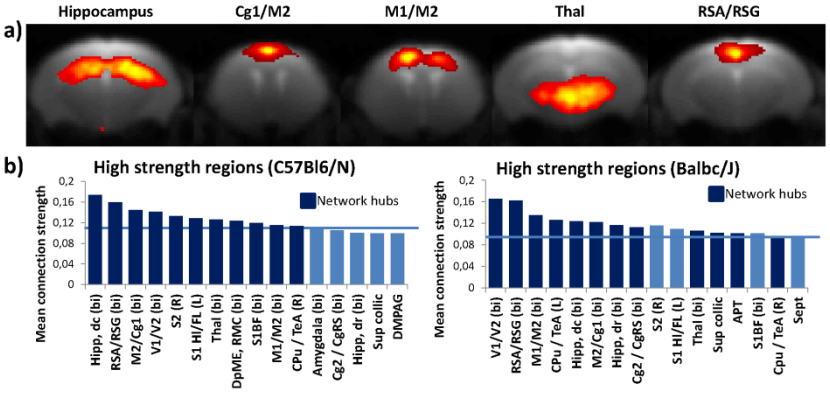
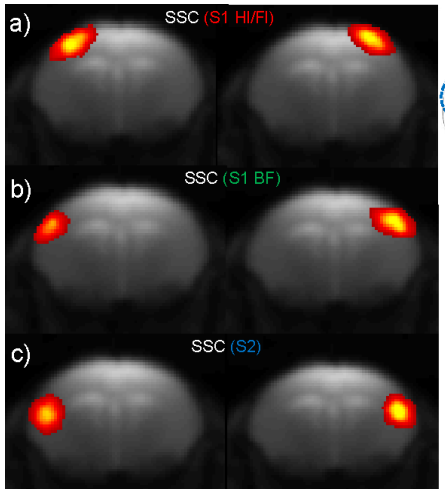


Figure 1: Comparative, strain specific classification of the FCN depending on the connection strength.
a) Exemplification of network hubs identified in both strains. The hubs were selected as the nodes (components) displaying both, above mean connection strength and number of statistically relevant connections.
b) Comparative display of the strong nodes observed in C57Bl6/n and Balbc/J strains, displayed in a decreasing order of their connection strength. Horizontal lines mark the mean strength in each population. Note the exclusion of the somatosensory areas (S2, S1 HI/FL and S1BF) as network hubs, in the Balbc/J group. Abbreviations: see Paxinos Mouse Brain Atlas [7].

Results and Discussion: Reliable and stable patterns of activation were obtained using 40 components group ICA (ICASSO), located in well defined cortical and subcortical brain areas. The clustering index I_q was higher than 0.8 for all 40 components, demonstrating this algorithmic reliability. 6 out of 40 identified components were discarded as being of vascular origin or artifactual. The relevant, influential brain regions were classified for each strain, using the strength and the number of relevant connections (Fig 1b). Regions with simultaneous above mean strength and mean number of relevant connections were considered hubs. Some of the hubs identified in both strains are shown in Fig. 1a. This includes, Hippocampus (Hipp), Cingulate cortex (Cg) and Retrosplenial (dys)granular cortices (RSA/RSG) as important areas of the limbic system, as well as motor cortex (M1/M2) or thalamus (Thal) which is also known as an important relay for structural connectivity. A first interesting between-strains difference was the inclusion of the somatosensory cortical (SSC) areas (segregated in S2, S1 HI/FL and S1BF) as hubs for the C57Bl6/N strain but their exclusion from the group of Balbc/J mice (see comparison of network hubs in Fig 1b). This exclusion was based on the lower nr of relevant connections (above mean) assigned for the Balbc/J SSC. We wanted to check further if the difference arises from decreased (statistically irrelevant) inter-hemispherical connectivity of the SSC areas or from reduced intra-hemispherical connectivity. The idea of modifications into the inter-hemispherical FC was also suggested by previous investigations of the structural networks[2,3] in Balbc/J mice, showing variations in the callosal inter-hemispherical pathway. Because most of the cortical networks identified with 40 components analysis included bilateral areas, we carried-out 100 component analysis, which segregated the cortical networks in unilateral patterns. This allowed checking the strength of connectivity between pairs of components such as: S1 HI/FI left vs. S1 HI/FI right; S1 BF left vs. S1 BF right; S2 left vs. S2 right. The results revealed significantly lower inter-hemispherical correlations between the primary SSC (S1 HI/FI, S1 BF), in Balbc/J population when compared with the C57Bl6/N strain (Fig 2). However, a stronger intra-cortical connectivity was assessed in this strain, suggesting a different assembly of the brain networks. This was also confirmed by slightly different partition of the FCN in modules, generated with graph theory analysis.



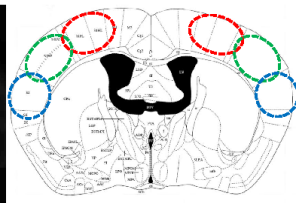


Figure 2: Segregation of the SSC cortex into functionally specific networks, using 100 components ICA. PC coefficients (r) were calculated for each strain:
a) S1 HI/FI left *vs.* S1 HI/FI right:
C57Bl6/N: r = 0.35 (significant)
Balbc/J: r = 0.05 (not significant)
b) S1 BF left *vs.* S1 BF right:
C57Bl6/N: r = 0.20 (significant)
Balbc/J: r = 0.06 (not significant)
c) S2 left *vs.* S2 right:
C57Bl6/N: r = 0.20 (significant)
Balbc/J: r = 0.18 (significant)

Conclusion: We depicted with rsfMRI inter-strain variations in the FCN of the mouse brain. Our results suggest a remodeling of the networks involving the primary SSC in the Balbc/J strain. Further analysis of our data would clarify the aspects of inter-individual variability within this mouse population.

References:
[1]Deco et al., NatRevNeurosc, 2011;[2]Harsan et al., Proc ISMRM 20, 2012;[3]Jacome LF et al., Autism, 2011; [4]Himberg et al., Neuroimage, 2004; [5]Rubinov, Neuroimage, 2010; [5]Paxinos and Watson, Mouse Brain Atlas in Stereotaxic Coordinates, 2001.