VOXEL-SCALE MAPPING OF THE MOUSE BRAIN FUNCTIONAL CONNECTOME

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Target audience. Researchers and clinicians interested in resting state fMRI connectivity and its aberrations in brain disorders.

Purpose. Resting-state BOLD functional magnetic resonance imaging (rsfMRI) has been widely applied to study functional segregation and integration in the human brain[1]. Network analyses of rsfMRI connectivity – a research effort also referred to as "functional connectomics" – have revealed the presence of functionally specialized sub-systems interlinked by a small number of highly-connected "hub nodes", serving as integrators of distributed neuronal activity. Aberrant connectivity of functional hubs has been described for several brain disorders[2]; however, whether these alterations are causative or epiphenomenal to brain pathology remains to be determined. The implementation of functional connectomic approaches in genetic mouse models could help pinpoint the elusive pathological significance of these connectional aberrations. To begin to address this issue, here we have applied a computationally-unbiased, threshold-free network analysis to map whole-brain intrinsic functional connectivity (i.e. the functional connectome) and identify functional modules and hubs in the mouse brain at a high-resolution voxel scale.

Methods. All experiments were carried out in accordance with Italian regulations governing animal welfare and protection. **Image data acquisition.** The procedure has been recently described[3]. Briefly, male C57BL/6J mice (N=41) were intubated and artificially ventilated; rsfMRI timeseries were acquired under controlled halothane anaesthesia (0.7%) on a 7T MRI scanner using a single-shot EPI sequence (TR/TE 1200/15ms, flip angle 30°, matrix 100×100, FOV 2×2 cm^2, 24 coronal slices, 0.50 mm thickness, 300 volumes). rsfMRI timeseries were motion corrected, spatially normalized, smoothed, regressed for motion traces and the mean ventricular signal, and band-pass filtered ($0.01 < f < 0.08$ Hz). The final spatial resolution was 0.2×0.2×0.5 mm^3. **Hub identification.** Time courses from all 16135 voxels in the brain tissue mask (excl. cerebellum) were extracted and Fisher's *r*-to-*z* transformed voxelwise correlation matrices were averaged across all animals and transformed back to *r* values to create the final connectivity matrix used to define the functional network, without any further arbitrary thresholding and/or binarization. The network was partitioned into modules by maximizing an asymmetric measure of modularity incorporating both positive and negative weights[4]. Hub nodes are commonly identified based on the strength of connections that nodes maintain and the distribution of these connections across modules. Accordingly, we identified as *global hubs* those nodes that showed disproportionately high connection strength[4] or connection diversity[4] (i.e. even distribution of connections across all modules). In addition, we identified as *module hubs* those nodes that exhibited high within-module strength[5]. To assess whether the identified hubs are preferentially and mutually interlinked, we analysed their connectivity relationships directly by considering the network comprising only overlapping connections between the hubs. Mean hub-hub correlation values were computed and the group-level significance of each connection was assessed using one-sample *t*-tests on *z*-transformed correlation coefficients, corrected for multiple comparisons using the Benjamini-Hochberg method and a false discovery rate of 0.01.

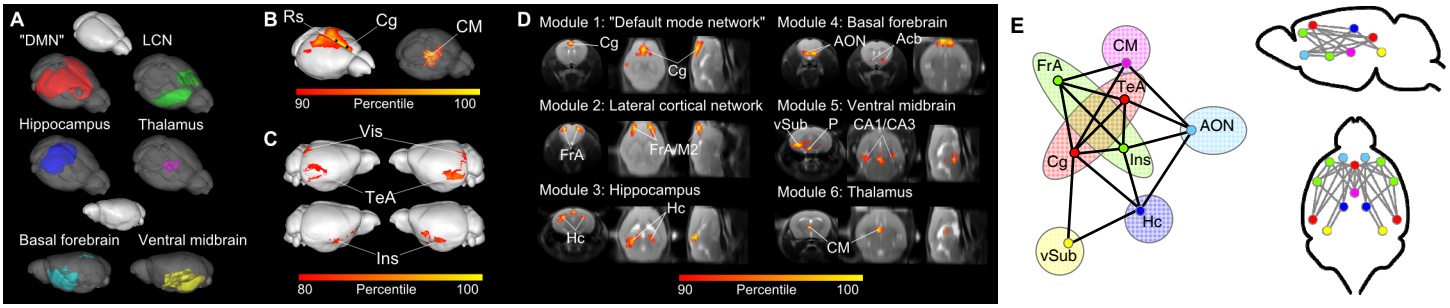

Figure 1. (A) A partition of the voxel-scale functional network into modules. (B) Global hubs – nodes exhibiting the highest connection strength (left) and diversity (right) in the mouse brain. (C) High connection diversity regions within the DMN and LCN modules. (D) Module hubs – nodes displaying the highest within-module strength values. (E) Graph representation of the connections surviving statistical thresholding and approximate locations of hubs. [AON, anterior olfactory nucleus; Acb, nucleus accumbens; CA1/3, CA1/3 fields of hippocampus; CM, central medial nucleus; Cg, cingulate cortex; FrA, frontal association cortex; M2, secondary motor cortex; Hc, hippocampus; Ins, insular cortex; P, pons; Rs, retrosplenial cortex; TeA, temporal association cortex; Vis, visual cortices; vSub, ventral subiculum]

Results. The voxel-scale mouse brain functional network was partitioned into six bilaterally symmetrical modules (Fig. 1A): a rodent homologue of the "default mode network" (DMN)[3,6], a lateral cortical network (LCN)[7], dorsal and ventral hippocampus, "basal forebrain" (striatal and septal regions, nucleus accumbens, anterior olfactory nucleus), "ventral midbrain", and thalamic areas. Foci exhibiting the highest strength nodes were located in several sub-regions of the DMN, including prefrontal, cingulate, and parietal association cortices (Fig. 1B, left). High connection diversity nodes were located in the thalamus (Fig. 1B, right), and within the cortex, in insular and temporal association areas (Fig. 1C). Module hubs within the DMN and LCN were localised in anterior cingulate and frontal association cortices, respectively (Fig. 1D). The identified hubs possess robust and preferential interconnections (Fig. 1E), suggesting that these regions act as a tightly interconnected sub-network within the mouse brain.

Discussion. Our findings describe topologically distinct neuro-functional modules of the mouse brain, including a DMN-like module, and identify a set of mutually-interconnected functional hubs that include well-characterised integrative cortical structures. Our data document the presence of evolutionarily conserved functional modules and integrative hubs serving as integrators of segregated functional systems in lower mammal species. Importantly, our approach also provides a fine-grained, threshold-free description of the mouse functional connectome that complements and integrates ongoing research in the large-scale connectional architecture of this species[8].

References. [1] Bullmore and Sporns. Nat Rev Neurosci. 2009; 10:186-198 [2] van den Heuvel and Sporns. Trends Cogn. Sci. 2013; 17:683-696 [3] Sforazzini et al. NeuroImage 2014; 15:403-415 [4] Rubinov and Sporns. NeuroImage 2011; 52:1059-1069 [5] Guimera and Amaral. J Stat Mech Theor Exp. 2005; P02001 [6] Lu et al. Proc Natl Acad Sci U S A. 2012; 109:3979-3984 [7] Schwarz et al. Brain Connect. 2013; 3(5):503-511 [8] Oh et al. Nature 2014; 508:207-214