Mapping the living mouse brain neural architecture: strain specific patterns of the brain connectional anatomy

Laura-Adela Harsan[1], Marco Reisert[1], Annette Merkle[1], Jürgen Hennig[1], and Dominik von Elverfeldt[1]
[1]*Medical Physics, Department of Radiology, University Medical Center, Freiburg, Germany*

Introduction: Mapping the brain connectivity fingerprints and the neural architecture is essential in neurology and experimental neuroscience because structural connectivity patterns could be related with functional and behavioral phenotypes[1]. However, a non-invasive detailed insight into the whole brain's axonal connectivity *in vivo* has only become possible since the development of diffusion based tractography. In mouse models, revealing strain related patterns in the brain structural connectivity or intra-strain variations of the brain wiring schemes have a tremendous importance, since these animals are used to answer questions relating to human neurological or neuropsychiatric disorders.

The primary goal of the present study was to probe, at microscopic level, the ensemble of anatomical neurocircuity of the living mouse brain, using diffusion fiber tracking, comparatively in BALB/cJ and C57Bl6/N mice. Particularly motivating was the investigation of the brain connectivity profiles of a population of BALB/cJ mice, a strain previously proposed as relevant model for autism behavioral endophenotypes[2]. Neural models of autism spectrum disorders have moved, in recent years, from a lesion model to a focus on abnormal brain connectivity[3]. Therefore, a detailed insight into the brain connectivity pattern of BALB/cJ mice might unveil relevant information for this still poorly understood brain disorder.

Materials and Methods:

8 weeks old BALB/cJ and C57Bl6/N male mice (n=10) were scanned under isoflurane anesthesia, using a 7T small bore animal scanner and a mouse brain adapted cryogenically cooled quadrature mouse brain resonator (Bruker, Germany). Mouse brain diffusion data was acquired using a 4-shots DT-EPI sequence (TR /TE = 7750 /26 ms; Δ=14 ms; δ= 4 ms). Diffusion gradients were applied in 30 non-collinear directions for a b factor of 1000s/mm^2. With the use of respiratory gating, the maximum acquisition time was of 25 minutes.

Diffusion data post-processing was performed using a FiberTool package developed in-house[4]. The analysis included the estimation of the fiber orientation distribution function (FOD) in each voxel via spherical deconvolution of the diffusion signal with a tensor response function. *Fiber tracking* was performed using a global optimization algorithm[5] that processed HARDI data and reconstructed all fiber bundles simultaneously, for the whole brain, without the requirement of defining seed or target regions. The fibers are built with small line segments that get bound together during the optimization and the connections are formed based on a probabilistic procedure. After the generation of sufficient number of streamlines passing a voxel at different locations, their density was used to construct a map that included as well the fiber directionality information[5,6].

High resolution mouse brain connectivity maps (hrCM) were generated by calculating and mapping the density of fibers in each element of a grid [6]. The grid size used for comparatively mapping the global BALB/cJ and C57Bl6/N mouse brain connectivity was tailored to match the thickness of the tissue section used for histological myelin staining (Fig 1). Therefore, the final reconstructed maps had a resolution (10 x 10 x 50 µm^3), ten times higher than the actual resolution of the acquired diffusion data (100 x 100 x 500 µm^3).

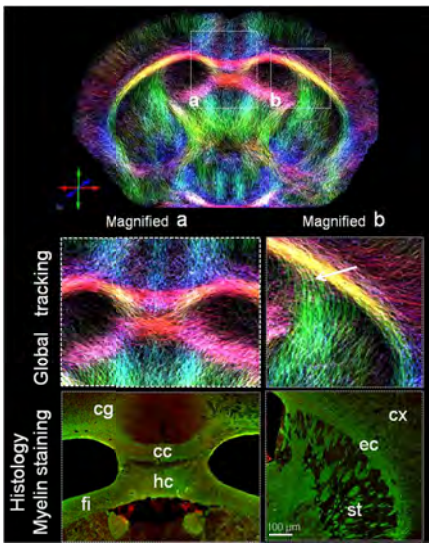

Figure 1: Representative high resolution brain connectivity map (hrCM - 10x10x50 µm³ resolution) of a living C57Bl6/N mouse brain compared with histological myelin staining (a and b – magnified views). Note the remarkable correspondence between the connectional anatomy features depicted with global diffusion tractography and the myelinated fiber tracts of the same animal (cc: corpus callosum, cg: cingulum, cx: cortex, ec: external capsule, hc: hippocampal commissure, fi: fimbria, st: striatum)

Results and Discussion:

Representative fine-grained topographic map of the living mouse brain connectional anatomy is shown in Fig 1. Remarkable correspondence between many of the structures visualized in hrCM of living mouse brain and those identified with myelin staining of brain histological sections is recognized (Fig. 1, a and b). This includes not only gross white matter connectivity profiles (i.e. corpus callosum, external capsule, fornix, fimbria), but also complex neural pathways passing white/gray matter transitions (Fig 1, arrow). Similarities in the striatal and cortical "texture" are also demonstrated.

Direct comparative visualization of global characteristics of the structural connectional anatomy in the BALB/cJ and C57Bl6/N is shown in Fig 2. Differences in the fibers density and shape of connecting structures were obtained between the two investigated mouse strains. However, the most prominent feature unveiled by hrCM was the substantial variability in the brain connectivity pattern of the BALB/cJ strain (Fig 2, A vs B). BALB/cJ mice show great *within-strain* variations in the interhemispheric connectivity trough the corpus callosum. Reduction of callosal interhemispheric connectivity, as seen in some of the BALB/cJ individuals (ie. Fig 2 A), is thought to contribute to the ethiology of autism disorders. BALB/cJ strain was previously proposed as a model relevant to autism behavioral endophenotypes, because, on average, juvenile BALB/cJ mice show low sociability in comparison with other inbred strains, included C57Bl6/N.

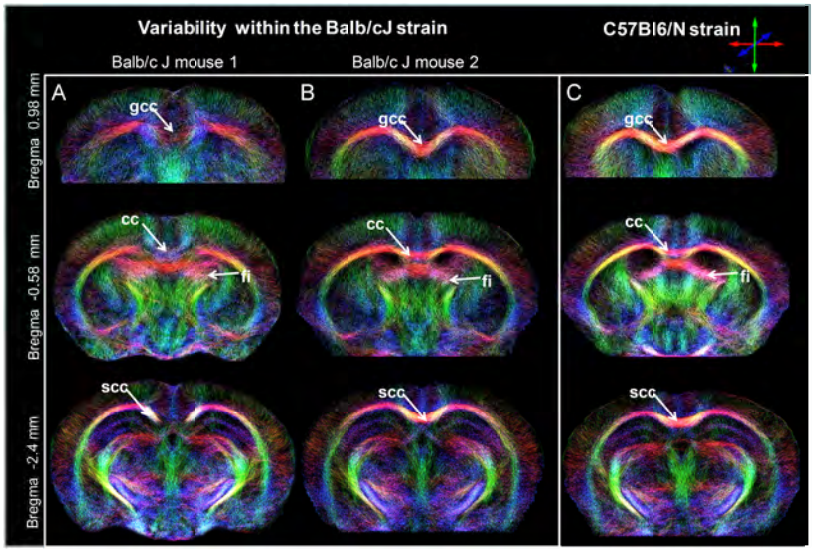

Figure 2: Example of high resolution brain connectivity maps (hrCM) of living Balb/cJ (A, B) and C57Bl6/N mice at different brain levels (bregma -0,5, -0.7 and -0.9).
A, B). Variability within the BALB/cJ strain. Reduced interhemispheric connectivity is seen in BALB/cJ mouse 1 (A) when compared with BALB/cJ mouse 2 (B), at different callosal levels (gcc: genu of the corpus callosum,, bcc: body of the cc, scc: splenium of the cc) **C)**. Overall view from the connectional architecture in a C57Bl/6N mouse, showing well defined connectional anatomy.

Conclusions: We depicted inter- and intra- strain variations in the general wiring scheme of the mouse brain. Exquisite brain anatomical details of the living mouse brain neuroarchitecture were revealed by combining the use of Cryoprobe technology for MRI data acquisition and a new global fiber tracking algorithm for post-processing. Our results hold the promise for the development of strain specific atlases of the whole living mouse brain *connectome*.

References: [1]Johansen-Berg, H. & Rushworth, M.F, Ann Rev Neurosc., 2009; [2]Jacome LF et al., Autism, 2011; [3]Vissers et al., Neurosci Biobehav Rev, 2011; [4]Kreher BW et al, MRM, 2008; [5]Reisert M et al., Neuroimage 2010; [6]Calamante F et al., Neuroimage 2011.