

Validation of In Vivo Mouse Brain Fiber Tracking with Correlative Axonal Tracing in Wild-Type and Reeler Animals

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Introduction:

Until very recently, the study of neural architecture using fixed tissue has been a major scientific focus of neurologists and neuroanatomists (1). A non-invasive detailed insight into the brain's axonal connectivity *in vivo* has only become possible since the development of diffusion tensor magnetic resonance imaging (DT-MRI) and fiber tracking (FT) (2). The method is now widely used for reconstructing *in vivo* the brain axonal pathways in humans and animal models. Although it seems clear that DT-MRI is able to reveal gross white matter connectivity, an accurate validation of the fiber tracking algorithms for identifying fine axonal trajectories is still lacking. In particular, validation of sensitive DT-MRI protocols and FT algorithms applied to depict *in-vivo* subtle connection pathways, in the small animal's brain would be of high value, given the great number of mouse models mimicking human degenerative disorders.

The primary goal of the present study was the rigorous validation of DT-MRI and deterministic and probabilistic mouse brain tractography with correlative histological axonal tracing. The study aimed to explore the use of *in-vivo* mouse brain tractography in identifying fine axonal pathways connecting areas of gray matter. DT-MRI data were tested for fine grained mapping of the thalamocortical connectivity, and more precisely the connectivity between the ventral posteromedial thalamic nucleus (VPM) and the somatosensory cortex, particularly, the highly organized rodent barrel field (SBF). 3D reconstructions of the thalamocortical projections derived from DT-MRI were co-registered and correlated with 3D reconstructions of the fibers labeled with *Phaseolus vulgaris*-leucoagglutinin (PHA-L) histological tracer, injected in the VPM of the same animal. Two aspects of the tractography were explored: the connection pathways and the correlation between the values derived from probability maps of connectivity and the density of axons labeled with the histological tracer. As a further refinement, impaired thalamocortical connectivity of the reeler mutant mouse was investigated with *in-vivo* tractography and axonal tracing.

Materials and Methods:

DT-MRI and FT: 7 wild-type B6C3Fe adult female mice and 7 *reeler* mutant mice were scanned using a 9.4T small bore animal Scanner (Bruker, Germany). Mouse brain DT-MRI was performed using a 4-shots DT-EPI sequence. Diffusion gradients were applied in 30 non-collinear directions for a b factor of 1000s/mm². With the use of respiratory gating, the maximum acquisition time was 2h. The diffusion tensor was calculated and estimates of the axonal fiber projections were computed by the fiber assignment by continuous tracking (FACT) algorithm. A brain mask was created and a tracking of the whole brain axonal pathways was performed. Seed points were placed in the VPM and SBF that were identified in high resolution T2-weighted images co-registered over the b0 image. The fibers connecting these areas and forming the thalamocortical projections were identified and displayed in 3D. A DT-MRI probabilistic approach (3), capable to determine in a statistical way the most probable neuronal pathway connecting two seed regions was further applied. The method required two steps: First, probability maps were generated separately for each seed region (VPM and SBF). The number of random walks was set to 1000 and maximum fiber length to 150 voxels. The second step combined the previously generated maps to derive the most probable direct pathway between the corresponding seed regions. The combined maps were scaled to the range between 0 and 1.

Axonal tracing: After the MRI procedure, PHA-L was stereotaxically injected in the VPM of the mouse brains. 24 h later the mice were perfused and 50µm brain sections were collected and analyzed for the histological tracing. A grid of 156x156µm² was placed on the histological tissue, matching the DT images voxel size and the labeled axons were counted in every 10th slice. Therefore for each MRI slice (500µm thick) the corresponding histological section was analyzed.

Results and Discussion: Thalamocortical pathways, identified *in vivo* with deterministic and probabilistic FT in wild type mice are exemplified in the Figure 1 (A, C, D). The fibers from the VPM are crossing the internal capsule (red arrow, Fig. 1A), they run tangentially at the interface of the cortex with the subcortical white matter and ascend to reach the target fields more superficially into the cortex. The probabilistic tracking show the same pattern of connectivity between the 2 selected seed points (Fig. 1 C, D). From the histological data (e.g. Fig. 1-G,H) axonal density maps were generated (Fig. 2, A and D) and co-registered (using landmarks) over the corresponding morphological MRI image and the probability maps of connectivity (e.g. Fig. 2, B, E). Image correlation algorithms were applied giving information about the overlap of the pathways derived from FT and histological tracing (Fig. 2, D, E, F). An example of such colocalisation is given in Fig. 2 and the calculated overlap in the exemplified images was of 81% and in general good agreement between results from the tractography and histological tracing was obtained. The described methodology was further applied for characterizing the thalamo-cortical pathways of the reeler mutant brain. The reeler mouse is a well characterized model of disorganized cerebral lamination. Due to the impaired neuronal positioning, the thalamocortical projections reconstructed in our study with *in-vivo* tractography and with axonal tracing were distorted, less compacted and thinner than normal. The fiber tracking pictorials (Fig. 1-B) are in agreement with our histological observation of poorly compacted axonal projections that penetrate the cortical plate and run up diagonally in the outer regions of the cortex. From these areas they descend to the deeper cortical planes where the target neurons wrongly end up their migration (Fig. 1-B). Additionally the probability maps of connectivity depict fine details of the cortical connectivity, similar with the labeling pattern seen in the cortical areas of mice injected with PH-L tracer (compare Fig. 1 –E,F,H). Interestingly, when comparing the probabilistic maps derived from wild type and reeler brains one could notice the high probability of connectivity indexes in the wild type somatosensory cortices at the level of cortical layer IV (Fig. 1-C-arrow). This is well correlated with the axonal tracing, the projection targets from VPM being represented by the neurons from cortical layer IV (Fig. 1-G-arrow). The probability maps of connectivity derived for the reeler (e.g. Fig. 1-E, arrows) show the termination fields in the more superficial layers of the cortex, as seen also on histological sections (Fig. 1-H-arrow).

In conclusion, the present work validate an *in-vivo* DT-MRI and FT protocol capable of identifying and characterizing the subtle connection pathways in the living mouse brain.

(1) Seung HS. Neuron 2009;62(1):17-29. (2) Jones DK. 2008;44(8):936-952. (3) Kreher et al., NeuroImage 2008;43 (1):81-89.

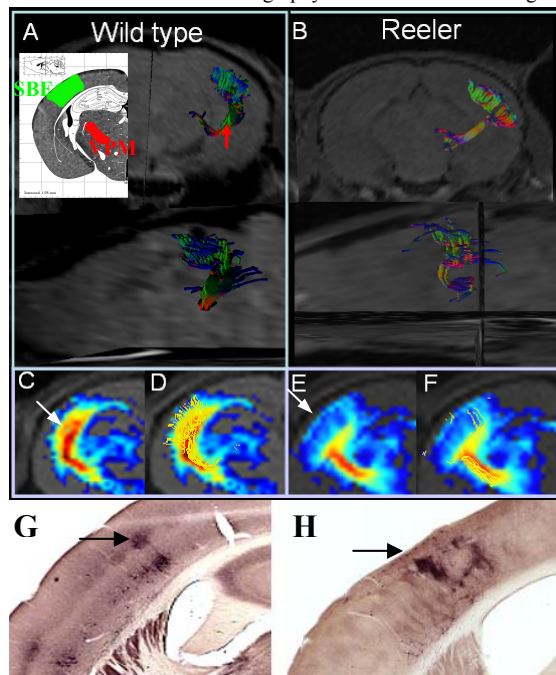


Figure 1: A, B. Thalamocortical projections of the wild-type and Reeler mice in frontal (top row) and lateral views. C, D, E, F. Examples of probability maps of connectivity between VPM and SBF in wild type (C,D) and reeler brain (E,F). D,F: Fibers crossing the plane of presented image and obtained with the FACT algorithm were superimposed on probabilistic maps. G,H. Histological sections exemplifying the target neurons in the SBF, labeled with PHA-L. G (wild type), F (reeler).

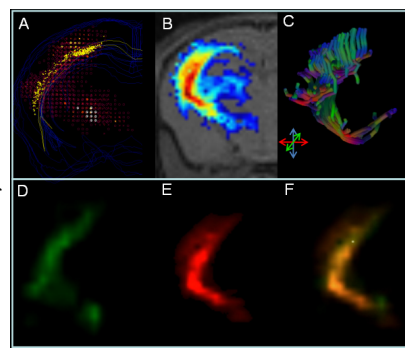


Figure 2: Different type of representations of the thalamocortical projections obtained in a wild type animal. A, D. Axonal density maps generated after counting the labeled axons in the histological sections. A. Overall view from all the analyzed sections (yellow spots-high density). D. Axonal density map generated from one single histological section. B, E. Probability maps of connectivity (scaled from 0 to 1) compared to the axonal density map showed in D. F. Colocalisation algorithms show good agreement between D and E (overlap of 81%). C. 3D reconstruction of the thalamocortical projections derived with the FACT algorithm.