Histological Validation of DTI using WGA-HRP in a Macaque

S. Peled[1], V. Berezovskii[2], P. Hendrickson[2], R. Born[2], C-F. Westin[3]

[1]Harvard Center for Neurodegeneration and Repair, Boston, MA, United States, [2]Department of Neurobiology, Harvard Medical School, Boston, MA, United States, [3]Laboratory of Mathematics in Imaging, Brigham and Women's Hospital & Harvard Medical School, Boston, MA, United States

INTRODUCTION

Since the pioneering times of Golgi and Cajal, tracing neural connections has been a challenge in neuroanatomy, with profound implications for the study of neural function and for the study of developmental and adult plasticity of the nervous system. The first generation of tract-tracing techniques based on intra-axonal transport - methods utilizing the uptake transport of horseradish peroxidase (HRP) - still rank among those most widely used in neuroanatomy. Today, we have an *in vivo* technique for gross fiber tract tracing - white matter tractography based on diffusion-weighted MRI data. As the sophistication of tracking algorithms increases, it becomes more and more essential to validate the resulting tracings. Here, we used Wheat Germ Agglutinin (WGA) HRP in a Macaque to create a "gold-standard" histological data set corresponding to acquired diffusion-weighted data. WGA-HRP is injected into live animals after which it is transported in both anterograde and retrograde directions along axons. After waiting a suitable amount of time, the animal is sacrificed and histological analysis takes place. The success of this method can be ascribed to several characteristics: It is fast and easy to implement, complicated injection apparatus is unnecessary, and the histochemical reaction products are visible under light and electron microscopes. The stained fibers can be followed through the brain to provide a basis for comparison with DWI-derived tracts computed for the same animal. The main advantages of the WGA-HRP method in the context of tracing validation are that it delineates a whole tract, and not just in-plane regional directions of white matter, as does silver staining, and that it yields points of insertion in the gray matter.

METHODS

Using procedures approved by the Harvard Medical Area Standing Committee on Animals, craniotomies were performed on a Macaque monkey, and 4% WGA-HRP was pressure-injected under microscopic stereotaxic guidance into primary visual cortex (V1), primary motor cortex (precentral gyrus) and somatosensory cortex (postcentral gyrus) on the left side. Four 0.25 microliter injections were made in V1, and five 0.2 microliter injections each in pre- and post-central gyri. Three days later the monkey was sacrificed. *Ex vivo* imaging of the fixed brain was performed at 4.7T using spin-echo DWI (30 directions, b-values of 1000 s/mm^2), with voxel dimensions 0.5x0.5x1mm^3 (*in vivo* DT-MRI data was also acquired prior to sacrifice but is not described here). After imaging, the brain was blocked into two parts to facilitate sectioning, with a concomitant tissue loss of approximately 600μ at the interface. Digital imaging during coronal sectioning in a cryotome was performed in order to capture the undistorted brain in a fixed coordinate system for subsequent 3D reconstruction, using a rigidly mounted Hasselblad camera with a Phase 1, 25 Mpixel, digital back. Histochemical analysis of the 80μ thin sections were performed on a subset of the sections which were then mounted on glass and scanned.

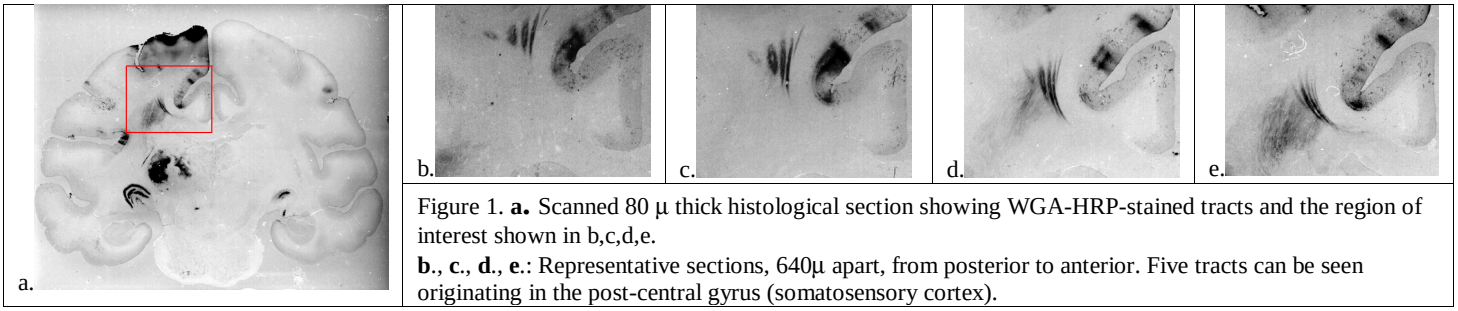

Figure 1. **a.** Scanned 80 μ thick histological section showing WGA-HRP-stained tracts and the region of interest shown in b,c,d,e.
b., **c.**, **d.**, **e.**: Representative sections, 640μ apart, from posterior to anterior. Five tracts can be seen originating in the post-central gyrus (somatosensory cortex).

RESULTS

Figure 1 shows glass-mounted histology sections of the Macaque brain. There are five converging trace lines resulting from the WGA-HRP being transported from the five locations of insertion in somatosensory cortex down to the corpus callosum (lower right in Figure 1.b-e). The WGA-HRP signal becomes much weaker upon entering the corpus callosum. Note the crossing tract that appears in Figure 1.d.

Figure 2 shows a 3D reconstruction of tracts calculated using a standard hyper-streamline tractography method that follows the principal diffusion direction from DT-MRI data originating in the somatosensory cortex. The tracts show convergence as they bend around the cingulate gyrus and divergence upon entering the corpus callosum. Such horizontal divergence of the tracts in the corpus callosum would cause the reduced concentration of WGA-HRP in each histological slice.

SUMMARY

We have obtained a unique data set that includes *in-vivo* DTI and 3D anatomical MRI, high resolution *ex-vivo* DTI, digital images of each section in the frozen undistorted brain, and WGA-HRP stained histological sections of the whole brain, of which a subset has been mounted and scanned. The tracts originating in the left somatosensory cortex calculated from DTI correspond extremely well with the histological reference. The data set will be used for tractography algorithm assessment, and for crossing tract elucidation.

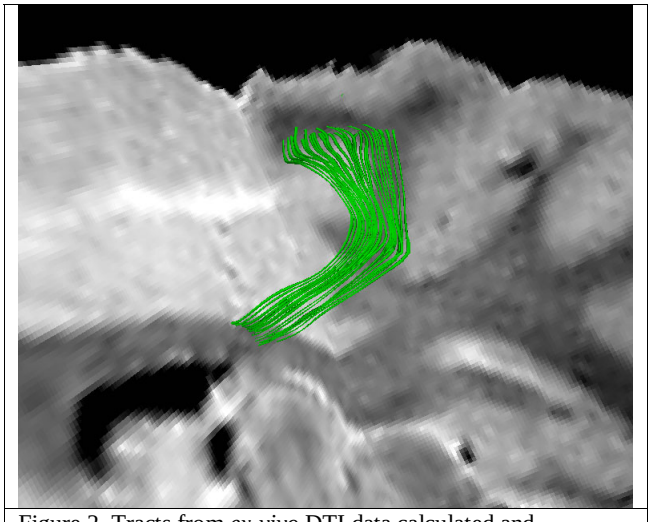

Figure 2. Tracts from *ex-vivo* DTI data calculated and displayed using 3D Slicer software (Surgical Planning Laboratory - BWH). Seed points were chosen in the white matter beneath the left somatosensory cortex.