33rd Annual International Conference of the IEEE EMBS
Boston, Massachusetts USA, August 30 - September 3, 2011

Tractographic Reconstruction Protocol Optimization in the Rat Brain *in-vivo*: towards a Normal Atlas

Maria Giulia Preti, Alessandro Di Marzio, Alfonso Mastropietro, Domenico Aquino, Giuseppe Baselli, Maria Marcella Laganà, Ileana Zucca, Carolina Frassoni, and Roberto Spreafico

Abstract—**The tractographic reconstruction of anatomical and microstructural features provided by Magnetic Resonance (MR) Diffusion Tensor Imaging (DTI) gives essential information of brain damage in several pathological animal models. The optimization of a tractographic protocol is undertaken in normal rats for the future construction of a reference atlas, as prerequisite for preclinical pathological *in-vivo* studies. High field, preclinical *in-vivo* DTI faces important difficulties relevant to Signal-to-Noise Ratio (SNR), distortion, high required resolution, movement sensitivity. Given a pixel-size of 0.17 mm and TE/TR = 29/6500 ms, b value and slice thickness were fixed at 700 s/mm^2 and 0.58 mm, respectively, on preventive *ex-vivo* studies. *In-vivo* studies led to the choice of 30 diffusion directions, averaged on 16 runs. The final protocol required 51 min scanning and permitted a reliable reconstruction of main rat brain bundles. Tract reconstruction stopping rules required proper setting. In conclusion, the viability of DTI tractography on *in-vivo* rat studies was shown, towards the construction of a normal reference atlas.**

I. Introduction

SMALL animal neuroimaging is gaining great attention in preclinical studies of neurological diseases. In the framework of Magnetic Resonance Imaging (MRI) techniques, Diffusion Tensor Imaging (DTI) permits the exploration of microstructural tissue features through the observation of water molecular diffusion, thus furnishing information about the anatomy, microstructural features, and damage of the main brain bundles, useful in several pathological animal models. Previous works of this group [1]-[4] faced the problems of diffusion sequence optimization and tractographic atlas construction in clinical DTI studies at 1.5 Tesla. However, though well established in clinical trials, this technique still requires the definition of proper protocols and normality references in preclinical small animal studies performed on high field scanners. This research line at a preclinical stage appears of importance, in order to help in defining the mechanisms involved in the initiation and development of the investigated disease. DTI is based on the estimate of the Diffusion Tensor (DT) in each voxel [5]-[7], which allows to determine the principal diffusion direction and the indices of Mean Diffusivity (MD) and Fractional Anisotropy (FA) [8]. DTI-based tractography permits the virtual reconstruction of the white matter (WM) fiber bundles *in-vivo*, following the principal diffusion direction; subsequent segmentation permits the extraction of diffusion parameters specific to any addressed bundle. Small animal studies require specific skills due to: small size and high required resolution, motion artifacts, Signal-to-Noise Ratio (SNR), and specific animal anatomy. Sensible improvements are offered by the use of high magnetic field, yet an accurate choice of the acquisition and processing parameters is needed, namely for *in-vivo studies*. Few studies reported DTI analysis in rodent brain in presence of stroke [9] and in developing stages [10]; tractography has been performed in mouse [11], [12] and in rat [13], [14]. To our knowledge, a single reference atlas reconstruction has been carried out, so far [15]; however, in mouse and by means of *ex-vivo* scans. The availability of reliable reference atlases for a given species is an important prerequisite for further pathological studies providing both bundle specific normality ranges of DTI parameters and bundle localization/segmentation through co-registration of the pathological brain on atlas.

The present work is preliminary to the production of a tractographic atlas of normal Sprague Dawley rats *in-vivo* and aims at the optimization of the scanning sequence parameters and at the definition of a suitable standard for the selection of the main rat bundles. Namely, sequence parameters such as the gradient direction number, repetition number, b-value, and voxel size are considered to optimize SNR and tractographic reconstruction. As to the latter, specific tuning of stopping rules is performed.

II. Methods

A. MRI acquisition

Diffusion Weighted Images (DWI) were aquired using a 7 T MRI scanner (Bruker Biospec 70/30 USR, Bruker, Ger).

Manuscript received June 21, 2011. This work was supported in part by Fondazione Cariplo, project New-Generation Fluorinated Materials as Smart Reporter Agents in 19 F MRI.

G. Baselli is with the Department of Bioengineering, Politecnico di Milano, Italy (corresponding author, phone: +39-(0)2-2399-3368; fax: +39-(0)2-2399-3360; e-mail: giuseppe.baselli@polimi.it).

M. G. Preti is with with IRCCS S. Maria Nascente, Fondazione Don Carlo Gnocchi ONLUS, Milano, Italy and with the Department of Bioengineering, Politecnico di Milano, Italy.

A. Di Marzio is with the Department of Bioengineering, Politecnico di Milano.

A. Mastropietro is with the Department of Bioengineering, Politecnico di Milano and Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

M. M. Laganà is with IRCCS S. Maria Nascente, Fondazione Don Carlo Gnocchi ONLUS, Milano, Italy.

D. Acquino, I. Zucca, C. Frassoni, and R. Spreafico are with the Fondazione IRCCS Istituto Neurologico Carlo Besta, Milano, Italy.

Surface RF coil was used for reception and volume RF coil was used for transmission. A BGA12-S gradient coil with diameter of 12 cm was used. The maximum gradient strength was 400 mT/m. DTI EPI spin echo sequences were acquired with the following parameter: TE/TR of 29/6500 ms, δ/Δ of 3/10 ms, pixel size of 0.176mm x 0.176mm, FOV= 3cm x 3cm, matrix= 170 x 170 and axial orientation.

The b-value and the slice thickness were first explored on an *ex-vivo* preparation (Sprague Dawley rat brain fixed in agar and 4% PFA) using the following values:

- *b-value* (600/900/1200 s/mm^2);
- *Slice thickness* (0.17/0.35/0.58 mm).

As a consequence (see results), b=700 and slice thickness of 0.58 mm were maintained for the subsequent *in-vivo* studies on a Sprague Dawley rat, 2 months, male. The rat was anesthetized with 2% isofluorane in O_2.

The following further parameters were varied and assessed for *in-vivo* optimization:

- *Number of gradient directions* (30, 126);
- *Number of runs for each acquisition* (4, 8, 16).

All experiments and procedure were approved by Ethic Committee (Fondazione IRCCS Istituto Neurologico "C. Besta", Milan, Italy) following the Ethical Use of Animals international guidelines.

B. SNR analysis

The well known relationship (1)

$$SNR \propto \mathrm{B}_0 \frac{voxelvolume}{noisevolume}\sqrt{T_{ro}} \qquad (1)$$

(T_{ro} is the total read out time over the runs number performed) establishes the tradeoff between SNR and other features such as small voxel size (important for tractographic reconstruction) and limited acquisition time compatible with *in-vivo* studies. Conversely, a good SNR is necessary both for DTI computations and the subsequent tractographic reconstruction, and is contrasted by the very nature of diffusion weighing based on signal attenuation.

SNR was estimated on the acquired images by using (2):

$$SNR = 0.7\frac{I_{OBJ}}{\sigma_{BKG}}. \qquad (2)$$

where I_{OBJ} is the mean intensity on a moving Region of Interest (ROI) of 10x10 pixels; the maximum value max(I_{OBJ}) on the entire object is taken for each image; σ_{BKG} is the standard deviation in 4 background ROIs, 10x10 each, fixed close to the image corners. The 0.7 coefficient is related to Rayleigh distribution [16]. The grand average through all slices and diffusion directions was used as SNR index of a specific acquisition.

C. DTI Analysis

FSL free package (http://www.fmrib.ox.ac.uk/fsl/) was used for eddy current correction. Affine registration using the FMRIB's Linear Image Registration Tool (FLIRT) was performed to register all b_0 images to that of the first run; the same registration parameters were applied to the relevant DWI of the same run. All the registered runs were than averaged using FSL and from the obtained set of DWI, the DT was estimated with Diffusion Toolkit software (http://www.trackvis.org) v0.6. Eigenvalues and eigenvectors were obtained for every voxel from the diagonalization of the DT and from them, the tensor scalar invariants FA and MD were computed. Deterministic tractography was performed with Diffusion Toolkit software using the Interpolated Streamline algorithm. Different angle and FA thresholds were used as stopping rules for the reconstruction of the different tracts. These thresholds were: FA=0.1/0.3/0.5 and Angle=10°/20°/30°/40°/50° for corpus callosum, cingulum, external capsule, caudate putamen, fimbria and optical nerve. The ROIs for tract segmentation were positioned on FA maps using Trackvis software (http://www.trackvis.org).

III. Results

As shown in Fig. 1, the higher is the adopted b-value, the better is the diffusion weighing. However, the SNR decrease prevented b-values largely higher than the lowest value experimented *ex-vivo*. For this reason, the b-value was fixed at 700 s/mm^2 in subsequent *in-vivo* studies.

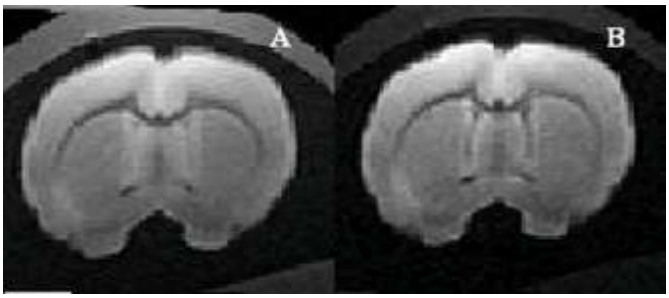

Fig. 1. Axial slices of rat brain DWI. A) b = 600 s/mm^2, SNR= 45; B) b = 1200 s/mm^2, SNR= 29.

As shown in Fig. 2, the minimum slice thickness of 0.17 mm with isotropic voxels, though optimal for DTI and fiber tracking [17], displayed a poor SNR, which is known to bias the DT estimate [18]. An increase of the slice thickness up to 0.58 mm was necessary; nonetheless, a successful DTI and tractography was verified, despite the uneven voxel aspect ratio.

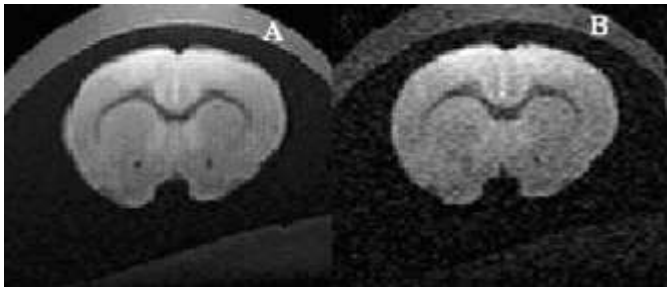

Fig. 2. Axial slices of rat brain DWI. A) Slice thickness=0.58 mm, SNR=42.77; C) Slice thickness=0.17 mm, SNR=12.40.

Passing to *in-vivo* studies, Fig. 3 compares the SNR with 30 directions/12 averaged runs to that with 126 direction/4 averaged runs scheme of acquisition given a similar total time of acquisition (T_{total} =28 minutes for 30 directions and 32 minutes for 126 directions). The improved raw data and previous results indicating that tensor DT reconstruction is not significantly improved beyond 30 directions [19] led us to choose 30 directions for further studies.

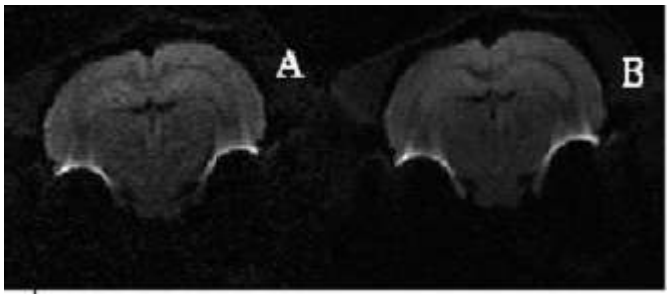

Fig. 3. Axial slices of rat brain b=0 images. A) DW acquisition scheme with 126 directions and 4 runs (T_{total}=32 minutes). B) DW acquisition scheme with 30 directions and 12 runs (T_{total}=28 minutes). It can be inferred from the two images that the 30 directions scheme has a better SNR than the 126 scheme, preserving a similar T_{total}.

SNR calculation on the image obtained by averaging different numbers of runs has been performed: as expected from (1), the higher is the number of runs (N_{avg}), the higher SNR results (Fig. 4). N_{avg} = 16 was chosen, since it provided a satisfactory SNR=43.61, preserving a still acceptable total scan time T_{total}=51 min, within the obvious constrains of *in-vivo* studies.

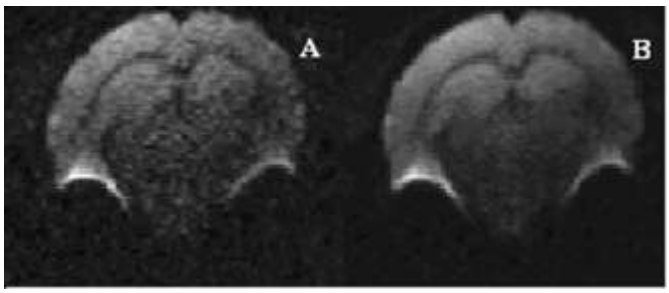

Fig. 4. DWI of rat brain in axial sections. A) N_{avg}=4, SNR=19,78. B) N_{avg}=16, SNR= 43.61.

Fig. 5 shows the presence of false tracts and biological aberration decreasing FA threshold (less than 0.5) and increasing angular threshold (more than 10°) for the corpus callosum. Same results have been obtained for all the other tracts but the optic nerve (optimal angle threshold = 40°).

Finally, 6 of the main rat brain tracts were reconstructed (*corpus callosum, fimbria, external capsule, caudate putamen, optic nerve* and *cingulum*), some of those are shown in Fig. 6.

IV. Conclusion

An acquisition sequence has been proposed with a total acquisition time (51 minutes) suitable for *in-vivo* acquisitions. The study has shown that a suitable parameter setting of a standard DTI sequence permits valuable tractographic reconstructions in control rat *in-vivo* studies aiming at a reference atlas for subsequent pathological studies. The scheme was verified to be reproducible and effective for the reconstruction of the main rat brain bundles *in-vivo*. Work is in progress as to inter-subject reproducibility, histological validation of tract selection, inter-subject normalization over a normal rat population for the construction of a tractographic atlas which is considered an essential prerequisite for studies on pathologic models.

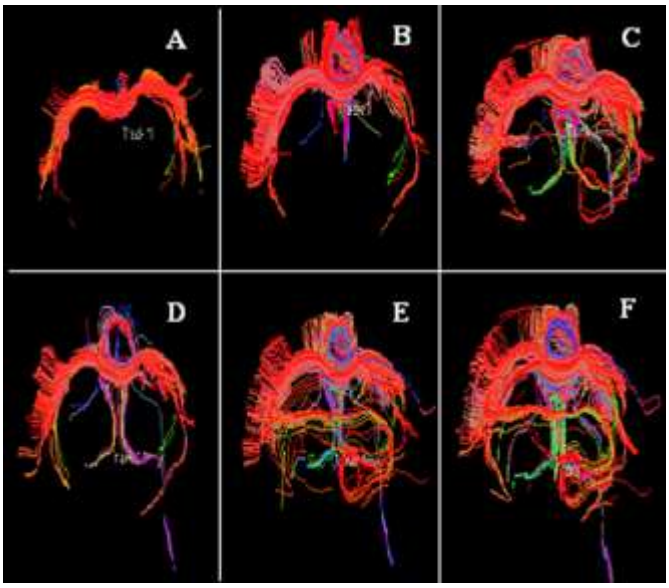

Fig. 5. Corpus Callosum reconstructions varying FA and Angle thresholds: A) FA=0.5, Angle=10°; B) FA=0.5, Angle=30°; C) FA=0.5, Angle=50°; D) FA=0.2, Angle=10°; E) FA=0.2, Angle=30° ; F) FA=0.2, Angle=50°. The figure shows the presence of false tracts increasing angle threshold and decreasing FA threshold.

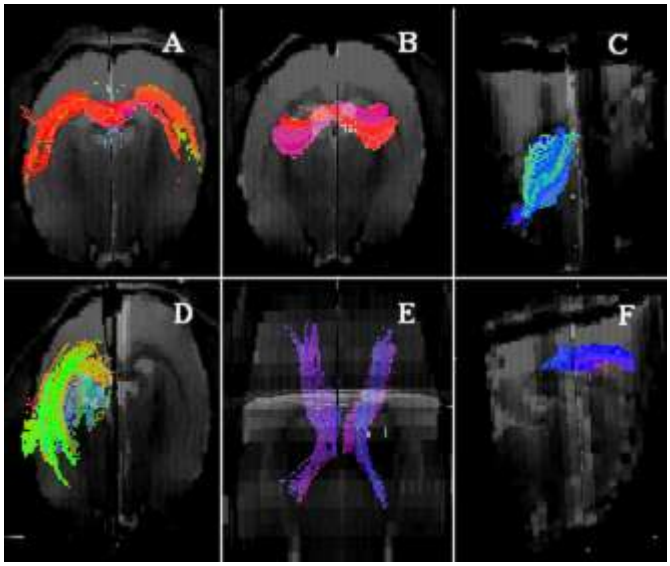

Fig. 6. Examples of some reconstructed tracts superimposed to b=0 images: A) Corpus Callosum, Axial view; B) Fimbria, Axial view; C) Right Caudato Putamen, Sagittal view; D) Left External Capsule, Axial view; E) Optic nerve, Horizontal view; F) Left Cingulum, Sagittal view. Fibers colors follow the sequent reference: red, left-right direction; blu, posterior-anterior direction; green, inferior-superior direction.

REFERENCES

[1] M. Laganà, M. Rovaris, A. Ceccarelli, C. Venturelli, D. Caputo, P. Cecconi, G. Baselli, "Atlas-based vs. individual-based deterministic tractography of corpus callosum in multiple sclerosis," *Conf. Proc. IEEE Eng. Med. Biol. Soc.* 2009; pp. 2699-2702, 2009.

[2] M. Laganà, M. Rovaris, A. Ceccarelli, C. Venturelli, Marini S., Baselli G, "DTI parameter optimisation for acquisition at 1.5T: SNR analysis and clinical application," *Comput. Intell. Neurosci.,* vol. 2010, pp. 1-8, Jan. 2010.

[3] M. Laganà, A. Ceccarelli, M. G. Preti, C. Venturelli, M. P. Sormani, R. Cavarretta, G. Baselli, P. Cecconi, D. Caputo, M. Rovaris, "Atlas-based vs. individual-based fiber tracking of the corpus callosum in patients with multiple sclerosis: reliability and clinical correlations," *J. Neuroimaging*, in press.

[4] L. Griffanti, F. Baglio, M. G. Preti, P. Cecconi, M. Rovaris, G. Baselli, M. M. Laganà, "Signal-to-Noise Ratio of Diffusion Weighted Magnetic Resonance Imaging: estimation methods and in-vivo application to spinal cord," *Biomedical Signal Processing and Control*, in press.

[5] P. J. Basser, J. Mattiello, D. LeBihan, "MR diffusion tensor spectroscopy and imaging," *Biophys. J.,* vol. 66, pp. 259-267, Jan. 1994.

[6] J. Mattiello, P. J. Basser, D. Le Bihan."Analytical expression of the b matrix in NMR diffusion tensor imaging," *J. Magn. Res.* ,vol. 108, pp. 131-141, 1994.

[7] D. Le Bihan, J. F. Mangin, C. Poupon, C. A. Clark, S. Pappata, N. Molko and H. Chabriat, "Diffusion tensor imaging: concepts and applications," *J. Magn. Reson. Imaging,* vol. 13, pp. 534-546, Apr. 2001.

[8] P. J. Basser and C. Pierpaoli, "Microstructural and physiological features of tissues elucidated by quantitative-diffusion-tensor MRI," *J. Magn. Reson. B,* vol. 111, pp. 209-219, Jun. 1996.

[9] G. Ding, Q. Jiang, L. Li, L. Zhang, Z. G. Zhang, K. A. Ledbetter, S. Panda, S. P. Davarani, H. Athiraman, Q. Li, J. R. Ewing and M. Chopp, "Magnetic resonance imaging investigation of axonal remodeling and angiogenesis after embolic stroke in sildenafil-treated rats," *J. Cereb. Blood Flow Metab.,* vol. 28, pp. 1440-1448, Aug. 2008.

[10] K. H. Bockhorst, P. A. Narayana, R. Liu, P. Ahobila-Vijjula, J. Ramu, M. Kamel, J. Wosik, T. Bockhorst, K. Hahn, K. M. Hasan and J. R. Perez-Polo, "Early postnatal development of rat brain: in vivo diffusion tensor imaging," *J. Neurosci. Res.,* vol. 86, pp. 1520-1528, May 15, 2008.

[11] R. Xue, P. C. van Zijl, B. J. Crain, M. Solaiyappan and S. Mori, "In vivo three-dimensional reconstruction of rat brain axonal projections by diffusion tensor imaging," *Magn. Reson. Med.,* vol. 42, pp. 1123-1127, Dec. 1999.

[12] J. Zhang, P. C. van Zijl and S. Mori, "Three-dimensional diffusion tensor magnetic resonance microimaging of adult mouse brain and hippocampus," *Neuroimage,* vol. 15, pp. 892-901, Apr. 2002

[13] D. Mayer, N. M. Zahr, E. Adalsteinsson, B. Rutt, E. V. Sullivan and A. Pfefferbaum, "In vivo fiber tracking in the rat brain on a clinical 3T MRI system using a high strength insert gradient coil," *Neuroimage,* vol. 35, pp. 1077-1085, Apr. 15, 2007.

[14] H. Chahboune, A. M. Mishra, M. N. DeSalvo, L. H. Staib, M. Purcaro, D. Scheinost, X. Papademetris, S. J. Fyson, M. L. Lorincz, V. Crunelli, F. Hyder and H. Blumenfeld, "DTI abnormalities in anterior corpus callosum of rats with spike-wave epilepsy," *Neuroimage,* vol. 47, pp. 459-466, Aug 15. 2009.

[15] T. Asanuma, S. Doblas, Y. A. Tesiram, D. Saunders, R. Cranford, J. Pearson, A. Abbott, N. Smith and R. A. Towner, "Diffusion tensor imaging and fiber tractography of C6 rat glioma," *J. Magn. Reson. Imaging,* vol. 28, pp. 566-573, Sep. 2008.

[16] S. B. Reeder, *Parallel Imaging in clinical MRI applications.* Chap 4: Measurements of Signal-to-noise ratio and parallel imaging. Springer Berlin Heidelberg, 2007, pp. 49-61.

[17] Y. Assaf and O. Pasternak, "Diffusion tensor imaging (DTI)-based white matter mapping in brain research: a review," *J. Mol. Neurosci.,* vol. 34, pp. 51-61, 2008.

[18] D. K. Jones and P. J. Basser, ""Squashing peanuts and smashing pumpkins": how noise distorts diffusion-weighted MR data," *Magn. Reson. Med.,* vol. 52, pp. 979-993, Nov. 2004.

[19] D. K. Jones, "The effect of gradient sampling schemes on measures derived from diffusion tensor MRI: a Monte Carlo study," *Magn. Reson. Med.,* vol. 51, pp. 807-815, Apr. 2004.