Validation of tractography against in vivo tracing in the macaque visual system – effect of distance correction

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Introduction: Diffusion MR imaging allows for the non-invasive *in vivo* examination of human brain connectivity patterns that have an important role in understanding brain function. Validation of this technique is important, but has proved difficult due to the lack of an adequate gold standard[1]. In this work, the macaque visual system is used as a model, in which due to an extensive literature of *in vivo* and post-mortem tracer studies, "true" connections are well-established[2]. In previous work from our group[4], we performed probabilistic tractography on diffusion imaging data from *in vitro* macaque brain, and comparisons were made between identified connections at different thresholds of connection strength, and known connections from the detailed visual system wiring map described by Felleman & van Essen[3]. In the present work we extend this to include comparisons from two macaque brains and explore the effects of streamline-length based correction of the distance bias of probabilistic tractography, in which the shortest, straightest paths are assigned higher connectivity values. Uncorrected tracking leads to a high percentage of false positive connections at short distance and false negatives (missed connections) at long distance.

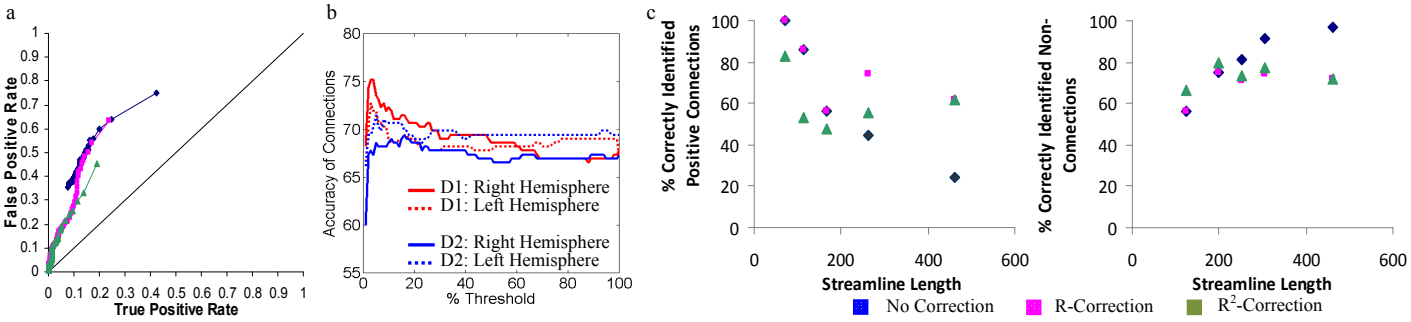
Methods: *Imaging*: Diffusion data were acquired in formalin-fixed post-mortem brains of two macaques. The first dataset (D1) was acquired in a *Macaca mulatta* on a 4.7 T Bruker BIOSPEC vertical bore scanner at the Max Planck Institute for Biological Cybernetics. A 2D spin echo sequence was implemented with TE = 78 ms, TR = 9 s, G_{max} = 47 mT/m, isotropic voxel resolution 0.8 mm, 61 non-collinear diffusion directions at b = 4,000s/mm^2 (Δ = 39 ms, δ = 31 ms), 7 at b = 0, NA = 4. The total imaging time was ~ 64 hours. The second dataset (D2) was acquired in a *Macaca fascicularis*. The brain was subjected to MCAo occlusion for 1 hour, even though there were no visible lesions on the diffusion tensor images. Acquisition was performed on a 4.7T Bruker Avance vertical bore scanner at the Athinoula A Martinos Center for Biomedical Imaging. A 3D spin echo EPI sequence was implemented with 8 shots, TE = 33 ms, TR = 350 ms, G_{max} = 380 mT/m, isotropic voxel resolution 0.43 mm, 120 non-collinear diffusion directions at b = 8,000 s/mm^2 (Δ = 18.8 ms, δ = 6.85 ms), 17 at b = 0. The total imaging time was ~ 27 hours. The imaging data were analysed with software developed and implemented in MATLAB. As previously described[4], constrained spherical deconvolution (CSD)[5,6] and model based residual (MBR) bootstrapping was then implemented on the acquired data, generating probability density functions in order to perform probabilistic tractography with the PICo[7,8] algorithm.

Cortical Parcellation: Using the non-linear warping function available as part of the Normalize tool in SPM5, the brain volume of the cortical partitioning scheme of Felleman and Van Essen[3] (FVE91) available as part of the Caret 5.5 software[9] for the F99UA1 rhesus macaque brain was spatially matched with the b=0 brain volumes of our datasets. The derived non-linear transformation parameters were then applied to the FVE91 cortical partitioning template.

Tractography: 22 cortical regions were identified in each hemisphere and were used as seed regions for probabilistic tractography using PICo with 1000 Monte Carlo streamlines initiated per voxel. For each dataset, the cortico-cortical interconnection (SCI) matrix of connectivity 'strengths', scaled between 0-100%, was created by measuring how many streamlines from a region passed through every other region. Using a range of thresholds for "accepted connection strengths" between 1 and 100%, comparisons were made with the Felleman and Van Essen atlas[3] and true positive (TP) and false positive (FP) rates were calculated. A measure of accuracy was determined, given as the percentage of correctly determined connections (including TP and true negatives (TN)).

Distance Correction: By recording the average length of the streamlines passing through each voxel, the lengths of the connection originating from each seed region was also estimated. As with the SCI matrices, by measuring the average length of the streamlines from a given seed region that passed through each of the other 21 regions, "length" of cortico-cortical interconnection (LCI) matrices were also generated for both hemispheres. The LCI matrices were used to compensate for previously reported[10] distance effects that influence probabilistic tractography results. In this work two methods of streamline length based correction were explored. First, as with probtrackx[11], the values in the SCI matrices were multiplied with the corresponding distance value in the LCI matrices (R-correction). Second, SCI matrices were multiplied by the square of the corresponding distance values in the LCI matrix (R^2-correction). To interrogate the success of the corrections, the % of correctly identified present and absent connections were calculated as a function of connection length by dividing all connections into 5 equal distance bins.

Results and Discussion:



The receiver operator curves (a) show reasonably good performance that is clearly above chance (black line) for all tested thresholds. The accuracy of connections (b) increases markedly up to an acceptance threshold of, respectively, 3% and 5% for datasets 1 and 2, beyond which accuracy is not greatly affected. At thresholds below the identified optima a large number of FP connections are present, whereas at higher thresholds the number of false negatives increases. 75% and 71% of connections were correctly identified in D1 and D2 respectively, showing good agreement between the results from each brain, despite quite different acquisition parameters. The fact that the results derived from datasets that were acquired using different protocols are so coherent also provides evidence that the results reflect true underlying connections and that they are not solely dependent on acquisition methods or as a result of noise. Our results demonstrate that a threshold of approximately 3-5% may be a good acceptance level for true connections when using PICo. The connectivity matrices (below) show good correspondence between the known connections from *in vivo* tracing (left) and the diffusion-based connections at the identified optimum acceptance thresholds (right). Some caution is needed in interpretation of these data as the *in vivo* tracer studies are assumed as a 'gold standard' measure of connections, which may not necessarily be true. This may also be apparent in our data, where certain connections were present in both datasets, but were absent in the Felleman and van Essen atlas. Our results therefore represent a lower boundary on the true accuracy of connection identification using tractography. As expected, without distance correction, the % correctly identified connections declines and the % correctly identified non-connections increases with increasing distance (c). Both the R and R^2-correction show good improvements in the % of correct connections identified at long distance (c), but the % of non-connections correctly identified declines, resulting in little notable increase in overall accuracy. Therefore, a more sophisticated correction method is needed. This work is the first to validate the performance of distance-based corrections against post-*in vivo* invasive tracer results.

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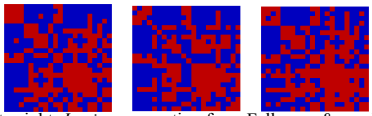

From left to right: *In vivo* connection from Felleman & van Essen[3], D1: thresholded without any correction, D1: thresholded using R-correction.

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