High resolution tractography in macaque visual system – validation against in vivo tracing

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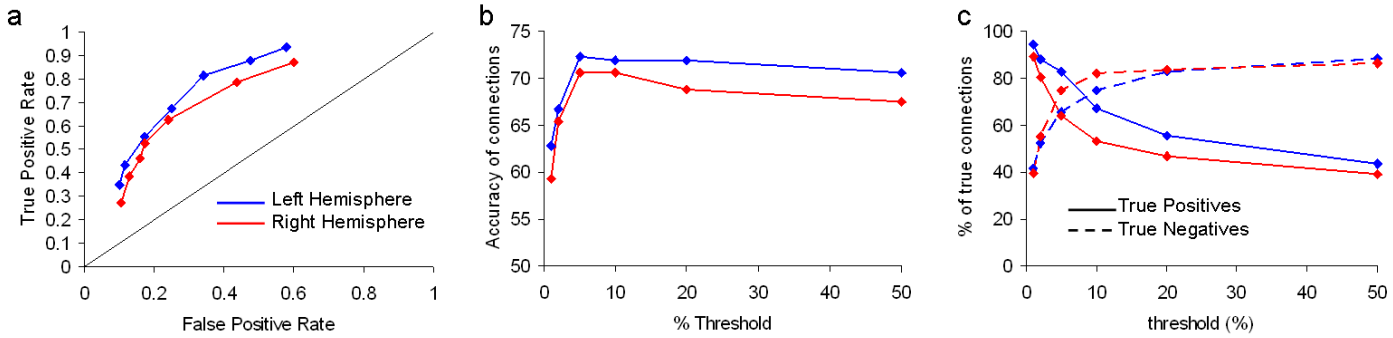
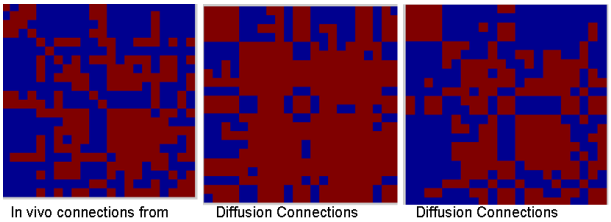
Introduction: Structural connectivity patterns are important for understanding brain function. Diffusion imaging offers the possibility of determining in vivo connections in the human brain. Validation of this technique is important, but has proved difficult due to lack of an adequate gold standard[1]. The aim of this work is to use the macaque visual system as a model, in which true connections are well-known due to many detailed in vivo tracer studies[2]. High angular resolution diffusion imaging (HARDI) of the post-mortem macaque brain, and a probabilistic tractography approach is used, and comparisons are made between identified connections at different thresholds of connection strength, and known connections from detailed visual system wiring map first described in detail by Felleman & van Essen[8]. 72% of connections were correctly identified.

Methods: *Imaging:* High angular resolution diffusion imaging (HARDI) MR data were acquired in a formalin-fixed post-mortem brain of a rhesus macaque (*Macaca mulatta*) on a 4.7T Bruker BIOSPEC vertical bore scanner. A 2D spin echo sequence was implemented with TE = 78ms, TR = 9s, G_{max} = 47mT/m, 104 × 94 imaging matrix, 58 contiguous slices, isotropic voxel resolution 0.8mm, 61 non-collinear diffusion sensitisation directions at b = 4,000s/mm^2 (Δ = 39ms, δ = 31ms), 7 at b = 0, NA = 4. The total imaging time was ~ 64 hours. To improve the signal-to-noise in the diffusion-sensitised images, for the purposes of tractography, we applied 5 iterations of 2D anisotropic diffusion smoothing[3] using ImageJ[4]. The imaging data was analysed with software developed and implemented in MATLAB
Constrained Spherical Deconvolution (CSD) and MBR Bootstrapping: We implemented CSD[5,6] on the acquired HARDI data. The response function was obtained from the simulation of a single diffusion tensor with fractional anisotropy of 0.8 and b = 1200s/mm^2. The fiber orientation distribution (FOD) function was generated with 45 spherical harmonics (l_{max}=8) and was then reconstructed at 8000 equidistant points on the sphere, within each voxel. A previously described model-based bootstrapping method[7] was used to generate probability density functions in order to perform probabilistic tractography for analysis within PICo[10,11] software.
Cortical Parcellation: We took the cortical partitioning scheme of Felleman and Van Essen[8] (FVE91) available as part of the Caret 5.5 software[9] for the F99UA1 rhesus macaque brain atlas. In order to use this cortical partitioning scheme in our dataset we applied non-linear warping to the anatomical MRI brain volume of F99UA1 to spatially match the brain volume of our dataset using the Normalize tool in SPM5. The transformation parameter file from this non-linear warping was then applied to the FVE91 cortical partitioning template.
Tractography: 22 cortical regions of interest were identified in the visual system of each hemisphere. Each of these regions in the spatially matched FVE91 template was used as a seed region for probabilistic tractography using PICo[10,11] in our dataset with 1000 Monte Carlo streamlines initiated per voxel. A cortico-cortical interconnection matrix was created by measuring how many streamlines from a specified cortical region passed through each of the other cortical regions. This gave us a matrix of "strengths" of cortico-cortical interconnection (SCI) on a scale of 0-100%. Connection strength was determined between all 22 regions within both the left and the right hemispheres, producing a total of 231 possible connections in each hemisphere. Comparing a range of thresholds for "accepted connection strengths" between 1 and 100%, comparisons were made to the Felleman and Van Essen atlas[8] (considering connections in both directions) and true positive and false positive rates were calculated. A measure of accuracy was determined, given as the percentage of correctly determined connections (including true positives and true negatives).

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 until an acceptance threshold of 5%, beyond which accuracy is not greatly affected. This is due to a step increase in the number of true negatives discarded as the threshold increases from 0 to 5% (c); i.e. at thresholds below 5% an unacceptable number of false positive connections are identified from the diffusion data. The true positive rate declines a little more slowly as the threshold increases (c). It was noted that the majority of false connections involved areas of higher level processing, particularly parietal and temporal regions. Similar results are found for both the left (blue) and right (red) hemisphere. Our results demonstrate that a 5% threshold may be a good acceptance level for true connections when using the PICo method, and ongoing work is investigating how this result is dependent on noise and other experimental considerations. The connectivity matrices (bottom left figure) show good correspondence between the known connections from in vivo tracing (left) and the diffusion-based connections at 10% acceptance threshold (right). Importantly, our results demonstrate that tractography can identify the majority of expected connections in the visual network and provide useful data to help define the limitations of the method. However, some caution is needed in interpretation of these data as it assumed that the in vivo tracer studies provide a 'gold standard' measure of connections, which may not necessarily be true. Our results therefore represent a lower boundary on the true accuracy of connection identification using tractography. This is the first time that anyone has been able to recreate the visual network at a level of detail approaching that described by Felleman and van Essen[8] based on invasive tracing data.



Connectivity matrices showing inter-connections between 22 regions of the visual cortex. Each column and row represents one region, red = connection, blue = no connection.

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