Comparison of probabilistic diffusion tensor tractography and histological tracer studies in the rhesus macaque

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Abstract. Examinations of probabilistic tractography have identified major tracts across the brain, but little has been done to compare them to well-established tracer studies. Therefore, diffusion tensor imaging (DTI) probabilistic tractography of white matter (WM) was performed on a DTI template generated from 271 rhesus macaques. Injection sites from tracer experiments in literature were chosen as seed regions from which to begin tractography. Results demonstrated the probabilistic connectivity maps obtained on the DTI template were largely consistent with connectivity maps from the tracer studies.

1 Introduction

While post-mortem examination of labeled white matter (WM) fibers remains the state of the art for examining structural connections throughout the brain, these studies are difficult to perform in humans. Diffusion tensor imaging (DTI) may be used to estimate the local orientation of WM fiber bundles in the brain (1). This directional information allows scientists to estimate and reconstruct the tract pathways noninvasively using tractography methods (2). DTI tractography is likely to further aid in our understanding of the connections within the human brain. Probabilistic tractography generates a spatial distribution of estimated tract connections and associated confidence.

Unfortunately, much of the extant knowledge about the ground truth for structural connectivity has been revealed by careful examination of injected tracers, designed to travel down the axons of WM, in rhesus macaque monkeys. Although DTI provides a unique opportunity to examine these connections in vivo within the human brain, little has been done to examine the precise limitation of this method as compared to the well-established tracer studies. Because differences between DTI-based tractography measures in humans and injected tracer studies in monkeys can be attributed to both technique and species differences, it will be important to study these differences within the same species. Therefore, we performed DTI tractography in a large sample
of rhesus monkeys and compared the resulting tracts to published results performed using more invasive methods.

A DTI brain template was created from 271 healthy young rhesus macaques (3). Here we performed probabilistic diffusion tractography in this template from seeds that correspond to the injection sites of reviewed tracer studies. The results are then used to analyze the similarities in connectivity images between our tractography and the tracer studies. Thus we have a method, within a single species, to examine how DTI tractography methods compare to ground truth anatomical data. We address possible limitations to these qualitative methods and outline future work in mapping the rhesus macaque’s brain.

In this paper we first discuss methods for aligning an atlas to the template, then using the CoCoMac (Collations of Connectivity data on the Macaque brain) database to identify brain regions to use as seeds and targets in a preliminary analysis. Next we attempt identification of specific injection areas from individual studies to use as seeds for probabilistic connectivity.

2 Materials and methods

2.1 Aligning the template to an atlas

ROIs from a digitized version of a rhesus brain atlas (4) were aligned to a rhesus T2-weighted MRI template (5). A rhesus DTI template (3) was created from 271 individual DTI scans (b = 1000 s/mm2, 12 non-collinear directions with one non-diffusion weighted image, acquisition repeated 6 times and averaged) with a native resolution of 0.547 x 0.547 x 2.5 mm. The template was created using DTI-TK, an advanced DTI spatial normalization and atlas construction tool (6) that incrementally estimates its displacement field using a tensor-based registration and optimizes tensor reorientation to preserve tensor properties (7).

The DTI template was resampled with a tensor-specific command (TVResample) to match the voxel dimensions of the T2 atlas, and then rigid registration was performed, all using DTI-TK software. The atlas-based ROIs were used to identify brain regions for both tractography seeding and reference points. In order to remove the effects of slight mis-alignment from rigid registration, and to avoid restricting the seed voxels to gray matter (GM), which would work poorly for WM tractography, atlas brain regions were always dilated once prior to use as seed regions or visual overlays.

2.2 Using the database to identify seed and target regions

The CoCoMac database (8) is an online resource compiling results from hundreds of tracer studies found in the literature. The user enters a brain region or abbreviation as a search query, and the result is a list of reported connections between that region and others. For each “seed” region used as a search query, all regions with qualifying connections documented were recorded, to create a list of “target regions”.

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Probabilistic tractography was performed using the “PICo” algorithm in Camino (9). For each voxel in the seed region, the algorithm uses DTI data to calculate the probability for each other voxel in the brain that it is anatomically connected to the seed voxel. The probabilities are averaged over all the voxels in the seed region, creating a map where the intensity at each voxel in the brain is proportional to the probability that it is connected to the seed ROI. For this study, the PICo algorithm was run with probability density functions (PDF’s) from the Bingham distribution, which allows elliptical probability density contours. Streamlines were generated with 2000 iterations at each voxel. A minimum fractional anisotropy (FA) value stopping criteria of only 0.05 was used, to allow the tracts to cross into GM if possible, but a curvature threshold was used to terminate streamlines if curvature between current and previous directions is greater than 80°.

After running probabilistic tractography on the seed region, both the target regions from the Paxinos-based atlas and the connection probability maps were applied as overlays on the template FA map. This enabled a visual comparison between connections identified by tractography and those identified in tracer studies. Because the tracer studies only report connections to GM regions, and tractography follows WM pathways, we do not measure correlation in the methods by direct overlap of labeled pixels, but rather by adjacency of connected WM paths to target GM regions. In a few representative studies, tractography was compared with tracer maps through similar brain sections.

2.3 Using specific reported injection sites as seed regions

To get a better sense of the finer accuracy of probabilistic tractography, we chose specific tracer studies and attempted to duplicate the injection site to use as a seed region. Since the literature usually defines injection sites only on illustrations of histological sections, it was impossible to perfectly replicate the injection sites, but illustrations provided clues to help us draw the seeds as accurately as possible. Gross slice placement could be ascertained from a sagittal image with coronal slice locations marked. Nearby brain regions labeled on the illustrations were cross-referenced with the Paxinos-based atlas regions, and the shapes of major sulci were visually compared, to identify the proper slice in which to place the seed region. Then seed regions were carefully drawn by hand to mimic the illustrations, and probabilistic tractography was run with them. A visual comparison of these probability maps and the study’s illustration of labeled cells was made. Many labeled regions could be identified on the probability maps.

The following studies were chosen:

- Schmahamann and Pandya, *Fiber Pathways of the Brain*, Case 21 (p. 263) - injection into ventral part of visual area 4 (V4)
- Distler et al. 1993, Case 1 - injection into TEO with labeled cells appearing in V2
- Cavada et al. 1989, Case 2 - injection into Broadman’s 7a (part of parietal areas PG and PF)
- Rockland et al. 1999, Case S43 - injection into pulvinar nucleus with labeled cells appearing in V2 and other visual areas
3 Results

3.1 Using the database to identify seed and target regions

This section displays results of probabilistic connectivity analysis performed on selected regions of the 3D Paxinos atlas. We display as “target regions” those regions of the atlas that were identified as connected to the seed region in the CoCoMac data.

Fig. 1 shows an example of a comparison between CoCoMac data and probabilistic tractography from our template. The seed region is Area 4 of the cortex. In this example we can see that the blue target regions lie adjacent to red regions of WM connectivity in most cases. In the more medial of the presented slices, it is evident that the deep GM structure VPL (ventral posterolateral thalamic nucleus) shows direct overlap with the probability map.

![Fig. 1. Every 3rd coronal slice of the DTI template FA map. Overlaid are: Area 4 seed in green, probabilistic connectivity map in yellow(high probability)-red(lower probability), CoCoMac-identified connected “target” regions in blue, and in areas of direct overlap with the connectivity map, appear purple.](image)

3.2 Using specific reported injection sites as seed regions

Next we attempted a more precise validation of tractography by visually duplicating illustrated injection sites from specific tracer studies. In each case we started by indentifying the regions named in the text as injection sites. In many cases, we used anatomical landmarks that were labeled in the illustration to help match the injection area as precisely as possible. We then used our reproduction of the injection site as a seed for probabilistic tractography, and used the illustrations of labeled cells from the studies as a qualitative comparison to the connection probability results.
Fig. 2. Left: An illustration from Distler et al. 1993 (10) of a tracer (WGA) injection into the TEO (black region) and the labeled cells (dots). Right: Probabilistic tracography results (yellow-red) using the TEO as a seed. Connections were found adjacent to region V2 (shown in blue as a guide) near certain areas in which the tracer study indicated labeled cells (green arrows).

Fig. 2 shows an early attempt in which we simply use the region TEO, or posterior inferior temporal cortex (listed as an injection site in (10)), as a seed. Connections were found adjacent to areas of V2 that were labeled in the paper’s illustration.

Fig. 3 shows a case in which we were able to recreate the injection site more accurately using an illustration (11) which labels several reference regions. The figure shows both the selection of seed region and the resulting probability map, which traces WM tracts that correspond well visually to tracts formed by labeled cells in the reference paper.
Fig. 3. Left: Selection of seed region on FA map. Top image from Cavada and Goldman-Rakic 1989 (11), indicates the injection site with the black region. The labeled regions DPI (dorsal prelunate cortex, modern V4), parietal area PO, and the calcarine sulcus (dark area on lower image) were used to determine the correct coronal slice in which to draw the seed region (green on lower image). Right: A more anterior coronal slice. Top image from Cavada shows labeled cells as dots, lower image shows FA map illustrating probabilistic tractography results from the seed region. Area V2 is show in blue as a reference. Tractography traces a path which replicates a path formed by labeled cells in the illustration, though the relative position in the image may be different (likely due to the effects of viewing a fixed vs. in-vivo brain slice).

Fig. 4 illustrates an even more precise selection of a seed region, reproducing the injection site of (12). The resulting probability map, shown in Fig. 5, corresponds qualitatively well to the portions of area V2 in which labeled cells were illustrated.
Fig. 4. a) Position in brain of pulvinar reference points. b) Left: Our drawing of the seed region (red) on our template FA, using reference areas PL (dark blue), PM (medium blue) and PI (light blue). Right: Illustration from Rockland et al. 1999 (12) specifying injection region. This illustration at a small scale allowed more specific seed placement than other studies. PL=lateral pulvinar, PM=medial pulvinar, PI=inferior pulvinar.
Fig. 5. a) Position in the brain of visual areas connected to pulvinar seed. b) Left: Results of probabilistic tractography in our template from the seed region (yellow-red). Area V2 (blue) is shown as a location reference. Right: Results from Rockland et al. (12) of the tracer injection from same seed. Black asterisks indicate areas of labeled cells. Black asterisks were placed in the left image to mark approximately the same brain area in our dataset. Short green lines delineate boundaries of area V2. FA images and illustrations are not scale matched, but slices were chosen based on landmarks and shapes of cortex.
Fig. 6. The DTI FA template (bottom) with a seed region (green) drawn based on Case 21 of Schmahmann and Pandya’s “Fiber Pathways of the Brain” (13) (top), the probabilistic tractography results from said seed (yellow-red) and the areas listed by Pandya as being directly connected to their seed tracer (blue). Adjacency is seen between many of the WM paths outlined in red and the GM regions outlined in blue. Additionally, the path traced by probabilistic tractography mimics the shape of the path of labeled cells in the illustration (green arrows).

Fig. 6 displays results of probabilistic tractography after best attempts to replicate injection/seed region in area V2 from Case 21 of Schmahmann and Pandya (13). The blue area indicates regions that were listed in the book as containing labeled cells. The connection probability map both runs adjacent to this blue area and follows a path visually similar to the provided illustrations, providing an extra layer of qualitative verification.
Fig. 7. Our DTI FA template (bottom) with a seed region (green) drawn based on Case 27 of Schmahmann and Pandya’s “Fiber Pathways of the Brain” (top), and the probabilistic tractography results from said seed (yellow-red). In this case the dotted areas in the top illustration representing labeled cells are not well-reproduced by the probability map.

Not all attempts at this kind of reproduction were as successful. Fig. 7 shows an example similar to the one in Fig. 6. In this case we reproduced an injection/seed region into Area 4 of the cortex. The resulting probability map recreates parts of the paths marked by labeled cells from the book’s illustration, but many other regions of heavily labeled cells are missing.

4 Discussion

These results provide a qualitative visual comparison between probabilistic tractography on a 271-subject rhesus DTI template and reported tracer studies. While this
type of analysis has its limitations, we believe it does demonstrate a degree of correspondence between tractography and tracer studies.

While it was expected that tractography might generate more false positives due to tracts mistakenly latching on to crossing fibers and following those paths, in reality there were many more false negatives. This helps to confirm that what is seen in tractography is not “wrong,” and also illuminates some of the specific shortfalls of this method.

There could be several reasons why probabilistic connectivity cannot fully reconstruct tracer maps. The diffusion tensor model cannot resolve crossing fibers. Tractography errors also increase with the length of the WM pathway. A subtle difference is that tractography estimates WM pathways, while CoCoMac database is defined by connections between GM regions. Thus it is difficult to assess the overlap of tracer distribution in WM with the tractography probabilities. The ability of tractography to reconstruct pathways at the GM/WM interface is also less predictable, thus reconstructions were assessed as being positive if the tracts either terminated in or were adjacent to the specific GM regions.

The results of this study are also limited by the alignment accuracy of the DTI template with the 3D Paxinos atlas. The alignment was done using only affine registration and manual adjustment. These templates were not created from the same animals and local anatomic inconsistencies and distortions would lead to misregistration.

The precision of region selection was also limited. When using the CoCoMac database, seed regions were selected based on regions of interest listed in literature – there was no quantitative data listing precise brain coordinates, and there was no cytoarchitectonic information available from the animals used in the template. It is likewise with target regions. Fig. 1 shows regions in red that were listed as being connected to Area 4 of the cortex. For larger extended regions, tracer studies did not always show connections to the entire target region, as in the inferior area of more posterior slices (lower rows of Fig. 1).

Tractography reconstructions were compared qualitatively with tracer schematics in Figures 2-7 to assess the general consistency between methods. In general, there was good correspondence in connectivity patterns although discrepancies were observed (Fig. 7). Tracer studies are considered the de facto ‘gold standard’ though there are limitations. These maps are generated from ex vivo brain sections thus post mortem extraction and sectioning will distort the anatomy. Further, these tracers mainly follow a specific pathway, but there is the chance for leakage into adjacent pathways. The tracer connectivity schematics are scanned and manually traced reproductions of visual stain or autoradiography mapping. All of these effects can lead to slight artifacts in the tracer ‘reconstruction’. Further, tracer studies in multiple animals demonstrate moderate variability, which may be differences in injection site and distribution and/or actual anatomical connections.

This work is unique compared to other studies that have performed comparisons of diffusion imaging techniques and radioactive tracer studies (14, 15), in that it investigates tract reconstruction from specific cortical areas, and compares tract endpoints.

It also validates use of tractography on a group template made of many individual animals. While others (16, 17) have compared histological results and probabilistic
tractography from the same injection/seed region in a single animal, we have provided more general results that work in a template made from a large population. This method cannot provide as precise a quantitative comparison of labeled voxels as previous work, but its qualitative results provide validation of tractography in population-averaged data sets, which are often used in neuroscience research.

Conversely, tractography was performed on an average DTI template from 271 animals, which will reconstruct a population averaged pathway reconstruction, but does not account for individual variation. The use of a group averaged diffusion tensor set for this application is novel. The averaging of this many brains increases the effective SNR though there is likely some blurring from the superposition of tensors. The animals in the template are also different than those in any specific tracer studies, thus it was not possible to do a one-to-one correspondence, here. Work is being carried out to move from this close visual analysis to a more generalized comparison to the CoCoMac database, similar to what has been done (15, 18), combining the novelty of our large-group dataset tractography data with quantitative information.

This study is an important step for evaluating tractography reconstructions and connectivity mapping from specific cortical areas. Despite the limitations with the techniques used, there was good qualitative correspondence between tractography and tracer studies. This is promising for using similar approaches for constructing a connectome of the rhesus macaque as well as the human. Future studies using high angular diffusion imaging methods for resolving crossing fibers will likely improve the reconstructions. The analysis will be extended to additional brain regions in the future.

References


6. Http://www.nitrc.org/projects/dtitk [Internet].


