Validation of High-Resolution Tractography Against In Vivo Tracing in the Macaque Visual Cortex

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Abstract

Diffusion magnetic resonance imaging (MRI) allows for the noninvasive in vivo examination of anatomical connections in the human brain, which has an important role in understanding brain function. Validation of this technique is vital, but has proved difficult due to the lack of an adequate gold standard. In this work, the macaque visual system was used as a model as an extensive body of literature of in vivo and postmortem tracer studies has established a detailed understanding of the underlying connections. We performed probabilistic tractography on high angular resolution diffusion imaging data of 2 ex vivo, in vitro macaque brains. Comparisons were made between identified connections at different thresholds of probabilistic connection “strength,” and with various tracking optimization strategies previously proposed in the literature, and known connections from the detailed visual system wiring map described by Felleman and Van Essen (1991; FVE91). On average, 74% of connections that were identified by FVE91 were reproduced by performing the most successfully optimized probabilistic diffusion MRI tractography. Further comparison with the results of a more recent tracer study (Markov et al. 2012) suggests that the fidelity of tractography in estimating the presence or absence of interareal connections may be greater than this.

Key words: diffusion imaging, macaque, tractography, validation, visual cortex

Introduction

Magnetic resonance diffusion images allow in vivo estimation of cerebral anatomical connectivity patterns using techniques such as tractography. However, there is still a need for an adequate gold standard against which such techniques could be validated (Hubbard and Parker 2009; Johansen-Berg and Rushworth 2009).

One approach to validation is to use computer-generated software phantoms (Gossel et al. 2002; Tournier et al. 2002; Lazar and Alexander 2003; Alexander 2005; Leemans et al. 2005; Watanabe et al. 2006; Descoteaux et al. 2007; Iturria-Medina et al. 2007; Sakai and Lowe 2007) or physical phantoms (Basser et al. 1994; van Doorn et al. 1996; Van Donkelaar et al. 1999; von dem Hagen and Henkelman 2002; Lin et al. 2003; Fieremans et al. 2005; Perrin et al. 2005; Yanasak and Allison 2006; Hubbard et al. 2015). These phantoms are relatively easy to define and manipulate by the user, but may grossly over-approximate the in vivo situation that is being simulated, as the complexities of white matter structures are difficult to reproduce. A priori knowledge of human neuroanatomy (Tournier et al. 2002; Abe et al.)
and circumstantial evidence from functional imaging studies (Guye et al. 2003; Toosy et al. 2004; Powell et al. 2006; Aron et al. 2007; Mao et al. 2007) and lesion (stroke and tumor) studies (Goss et al. 2002; Mori et al. 2002; Newton et al. 2006; Schonberg et al. 2006) are also valuable additional forms of validation (Hubbard and Parker 2009). Comparisons with such studies have highlighted the various attributes and pitfalls of different classes of fiber tracking methodologies.

Animal models provide a third avenue for validation. Within this context, the quantity and reliability of tracer data derived from macaque brains, the phylogenetic proximity of macaques and humans, and the ability to acquire data using a comparable imaging protocol make macaques a valuable animal model for validating tractography techniques. Parker et al. (2002) provided the first diffusion-weighted imaging comparison of the macaque and the human brains using fast marching tractography, based on diffusion tensor information. Subsequent comparison work included use of the q-ball fiber orientation estimation technique to enable tractography (Tuch et al. 2005), and investigation of macaque brain connectivity patterns (Croxson et al. 2005; Dauguet et al. 2007). However, although these studies showed to some degree the similarity of tractography output with the expected connection information, they did not explore the influence of the range of experimental tractography variables, such as trajectory curvature limits, which restricts the maximum angle through which paths can propagate, and fractional anisotropy (FA) constraints designed to avoid propagation into gray matter. Such variables have been shown (Jones 2010) to lead to variations in the extent and strength of derived pathways. Improvements in MR scanner and computer hardware and processing techniques in the last decade have also allowed the production of higher resolution and signal-to-noise data for MRI tractography, and a resultant need to evaluate the abilities of more recently developed fiber tracking methodologies (Dyrby et al. 2007).

Many invasive tracer studies have characterized the interconnections of the macaque visual system in detail, making it an appropriate model with which to assess the accuracy of tractography outputs (Van Essen et al. 1992). A detailed wiring map of the interconnections in the macaque visual system was first described by Felleman and Van Essen (1991; FVE91). Hence, via comparison with this reference system, diffusion MR images of the macaque brain can be used as a test-bed to validate the output of different tractography approaches between visual cortical regions. Therefore, the aim of this work is to quantify the accuracy of connections identified using MR diffusion-imaging-based tractography in the macaque visual system by comparison with known connections attained from previous invasive tracer studies. We use a probabilistic tractography approach to identify probabilistic connection “strengths” (streamline counts) between visual cortex regions of interest (ROIs) in 2 postmortem macaque brains. Comparisons with the anatomical connections of the FVE91 wiring map allow us to determine the optimum threshold of acceptance of streamline counts, and the accuracy of the tractography method. We assess the effect of distance correction, trajectory curvature and FA constraints on accuracy.

Materials and Methods

Image Acquisition

MR high angular resolution diffusion imaging (HARDI) data were acquired in formalin-fixed postmortem brains of 2 rhesus macaques.
is used to drive the tractography algorithm and that poor matching to the actual gray matter could negatively impact the analysis, the quality of the subject-specific region placements was then manually assessed through a slice-by-slice examination. Wherever required, ROIs were amended and repositioned to correspond to expected cortical landmarks (Saleem and Logothetis 2006), ensuring that they encompassed the gray matter only.

Tractography
Twenty-two cortical ROIs were identified in the visual system within both hemispheres of the 2 datasets, allowing us to perform intrahemispheric tracking. As the MRI measurements for D1 were obtained using a surface coil placed over the occipital lobe, we restricted our study to the visual system in both data sets. Each of the visual regions in the spatially matched FVE91 template was used as a seed region for performing probabilistic tractography using PICO (Parker and Alexander 2003; Parker et al. 2003) with 1000 Monte Carlo streamline propagations initiated per voxel of each seed region. For each dataset, a cortico-cortical interconnection matrix was created by measuring how many streamlines from a specified cortical region reached each of the other cortical regions. There was an additional step to impose symmetry: The connections were measured from each A → B pair and from B → A and the maximum number of streamlines from the 2 measurements was taken to be the value of connection. This gave us a symmetrical matrix of “strengths” of cortico-cortical interconnection (SCI) on a scale of 0–100% of initiated streamlines. Connection strengths were determined between all 22 regions within both the left and right hemispheres.

Comparison with In Vivo Tracing Data
The SCI matrices were compared with the interconnections described in FVE91, which are based on in vivo tracing results in various species of macaque including M. mulatta and M. fascicularis (Felleman and Van Essen 1991). True positives (TPs) were the connections established in FVE91 with high confidence (in either the forward or reverse tracing direction) and true negatives...
The symmetric gold standard connection matrix with regions in an alphabetical order, where white indicates a true connection, black no connection, and gray indeterminate. (b) The wiring diagram representations of the in vivo connections from Felleman and Van Essen with labels for different regions as defined in that work.

(TNs) the connections for which good experimental evidence of no connection existed. Potential connections for which the evidence was deemed unreliable in FVE91 (due to conflicting evidence or lack of findings for that connection) were discounted from our “ground truth” connection matrix (Fig. 2). We use the term “ground truth” not to imply that this matrix definitively represents true connections (as there are limitations to the accuracy of the invasive tracing data), but rather to imply that this matrix was used as a baseline against which the tractography findings were compared.

The accuracy of tractography-derived connections was calculated as the percentage of correctly determined connections (including TP and TN):

\[
\text{Accuracy} = \frac{100 \times (TP + TN)}{TP + TN + FP + FN}.
\]

where FP is the number of false positive connections and FN false negatives. Accuracy was calculated at every step increase of 1% (between 0 and 100%) in the acceptance threshold value applied to the SCI matrices. If the SCI is above this threshold, then the connection is deemed to be present. Receiver operating characteristic (ROC) curves were also generated by plotting the TP rates against FP rates, where TP$_{rate}$ = TP/(TP + FN) and FP$_{rate}$ = FP/(FP + TN). Finally, % TP was calculated using 100 × TP/(TP + FP) and % TN by 100 × TN/(TN + FN).

Some connections were found to be present in all 4 hemispheres of the macaque tractography data but not present in FVE91, and other connections were present in FVE91 but consistently absent in the tractography data. We thought it possible that these tractography connections that were classified as FPs or FNs may in fact be correct. We considered the accuracy of FVE91 for these connections by comparison with information provided by a further, recently published, quantitative tracer study (Markov et al. 2012), which reports an enhanced description of pathways within the visual system. Despite differences in the partitioning schemes of Felleman and Van Essen and the Markov analysis, we were able to identify analogous regions: visual areas 1,2, visual areas V3a, visual areas 4, V4 transitional, middle temporal, lateral intraparietal, medial intraparietal, ventral intraparietal, and posterior intraparietal were considered to be the same in both schemes, and further regions were identified with different naming conventions: posterior inferotemporal ventral (FVE) = TEO(M); posterior inferotemporal dorsal (FVE) = TEOm(M), central inferotemporal ventral (FVE) = TEpd(M), central inferotemporal dorsal (FVE) = TEp(M), anterior inferotemporal ventral (FVE) = Tav(M); anterior inferotemporal dorsal (FVE) = Tadv(M), PO(FVE) = V6(M), and V6a(M) combined, MST(FVE) = medial superior temporal, lateral (M) and MSTd(M) combined, and V3(M) encompassed V1 and ventral posterior in FVE. There was no analogous region in the Markov scheme for the region defined as ventral occipitotemporal in the Felleman and Van Essen scheme.

The Effect of Distance Correction, Curvature, and FA Constraints

By recording the average length of the streamlines leaving each seed voxel, the lengths of the connection trajectories originating from each seed region were estimated. As with the SCI matrices, symmetric “length” (in mm) of cortico-cortical interconnection (LCI) matrices were generated for both hemispheres in the 2 datasets; the larger of the lengths measured between 2 regions was used to define the connection in question. The LCI matrices were used to compensate for previously reported (Tomassini et al. 2007; Morris et al. 2008; Jones 2010) distance effects that influence probabilistic tractography results. Two methods of streamline length-based correction were explored. First, as implemented in FSL’s probtrackx (Behrens et al. 2007), 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 (R²-correction). To interrogate the success of the corrections, the TP and FP rates were calculated as a function of connection length by dividing all connections into bins, by ordering the connections according to length and placing an equal number of connections in each bin.

We also considered the effects of other constraints and optimizations that are commonly used in tractography experiments. The tractography experiments described above were repeated in both hemispheres for both datasets using 4 different FA streamline propagation termination thresholds of 0, 0.1, 0.2, and 0.3. These values are based on recommended values that were used in other studies (Kunimatsu et al. 2004; Stadlbauer et al. 2007; Parizel et al. 2007) and are founded upon considerations of the selection of a threshold that distinguishes gray and white matter. As our tracking start and termination regions lie within cortical gray matter, where the FA values may be lower than the thresholds used (i.e., 0.1, 0.2, and 0.3), the use of FA threshold values as streamline propagation termination constraints may end the
tracking process before tracking has left the seed mask. To allow for the streamlines to reach the white matter of the brains before the FA termination constraint initiates, a cortical gray matter mask was used to specify regions in which this constraint was not employed. These masks were also used at the far end of the streamlines where the paths penetrate the gray matter. The cortical gray matter mask was generated by combining all the cortical regions derived from the cortical partitioning scheme of FVE91. This was then warped onto the cortex in each dataset.

Another constraint that was explored was the use of curvature thresholds. This constraint is typically employed to allow for the expectation that, in white matter, at a voxel resolution, sharp changes in the direction of fiber pathways are not expected (Schmahmann et al. 2007; Wakana et al. 2007; Behrens and Jbabdi 2009). To test the effects of curvature constraints, curvature-based termination values of 70, 80, 90, and 180° were separately used as constraints at each step of the streamline propagation process.

**Results**

**Accuracy of Connections**

The ROC curves (Fig. 3A) for each hemisphere in each data set show performance that is clearly above chance (black line) for all tested thresholds of SCI. Figure 3B shows the effect of the acceptance threshold for SCI on connection accuracy, which is optimum between 2% and 5% for both datasets. Above this optimum threshold only the strongest connections are accepted, leading to an increase in the percentage of TP connections (Fig. 3D), but a decrease in the percentage of TNs (Fig. 3C) as weaker connections are missed (more FNs). Below the optimum threshold, progressively weaker connections are erroneously accepted (more FPs), reducing the percentage of TPs (Fig. 3D) but increasing the percentage of TNs (Fig. 3C). Average accuracies of 77% and 70% of connections at the optimum thresholds were found in D1 and D2, respectively (Table 1), showing good agreement between the results from each brain, despite quite different acquisition parameters.

The connection matrices (Fig. 4) show good correspondence between the known connections from in vivo tracing and the diffusion-based connections at the identified optimum acceptance thresholds for each hemisphere. The majority of FN connections were long range (Table 2), involving connections between different lobes. This may be explained by the inherent bias of tractography toward the shortest pathways. FP connections tended to be shorter range, with half being within the same lobe (Table 3). These “false” connections were compared with the results of a more recent quantitative tracer study (Markov et al. 2012) that has identified a number of additional pathways, as indicated by the footnotes “a” and “b” in Tables 2 and 3. Markov tested 9 of
the 18 connections identified as FPs relative to the FVE91 results, and identified 8 previously undocumented true connections and confirmed 1 nonconnection. These newer results are clearly in stronger agreement with the tractography results than the FVE91 results. However, Markov also tested 10 of the 40 FNs (Table 3) and confirmed the existence of 8 connections that were not found with tractography along with 2 connections that were confirmed to be absent.

The Effects of Distance Correction

Figure 5 shows the results of the distance correction methods on accuracy (compare with Fig. 3). Without distance correction, both the TP and FP rates decline with increasing distance away from the ROI seed point (Fig. 6). Both the R and R²-correction show some improvements in the TP rate identified at long distance (Fig. 6A), but the FP rate also increases (Fig. 6B), resulting in little notable increase in overall accuracy of the identified connections (Table 1).

The Effects of Streamline Curvature Termination Constraints

Curvature constraints appear to have no clear effect on the accuracy of the results (Table 1). Although the use of a curvature threshold increases the percentage of identified TNs, it also decreases the percentage of identified TPs.

The Effects of Variations in FA Termination Constraints

The use of higher FA termination thresholds appears to have a negative effect on the accuracy of the tracking results (Table 1), that is, when the FA threshold is increased, the level of accuracy of the tracking results is reduced. These results suggest that under the experimental conditions used in this work, there is no justification in using any FA threshold to terminate tracking.

Discussion

Our results demonstrate that a threshold of approximately 2–5% is a good acceptance level for SCI when using probabilistic tracking methods such as PiCo. We were able to achieve a tractography-based connection accuracy of 77% in D1 and 70% in D2 relative to the connections that were identified by Felleman and Van Essen. The difference in accuracy found between D1 and D2 is most likely to be due to differences in the data acquisition protocols and the fact that D1 and D2 are from different subspecies of macaque. Considering the uncertainty of false connections in the Felleman and Van Essen work, our results reflect the lower limit of accuracy.
Indeed, further comparison with the results of a recent quantitative tracer study (Markov et al. 2012) supports this conclusion (Tables 2 and 3). Their results suggest that nearly all of the consistent “FP” connections identified by our tractography experiments are true connections, but that the majority of consistent “FNs” are likely to be truly missed connections. This reflects the limited sensitivity of tractography, which is unlikely to be able to reproduce small, fine, or dispersed connection pathways. Furthermore, tractography is thought to be biased toward terminating on gyral crowns rather than on sulcal walls (Jbabdi and Johansen-Berg 2011), which may have contributed to the large number of FNs, which tend to involve small areas in the sulci (Table 2). This suggests that the true accuracy of the tractography results may be greater than suggested by comparison with the Felleman and Van Essen results, and also that a lower threshold of acceptance for the tractography results may be appropriate to capture more of these missed connections. Use of the CoCoMac (http://cocomac.org) database, which is a more up-to-date and comprehensive collection of macaque invasive tracer information, may also offer a better test of accuracy than the Felleman and Van Essen map.

Our analysis did not take into account differences in the physical diameter of the tracts, or the fact that some connections may be associated with subregions within our cortical regions. Both effects are likely to lead to reduced sensitivity of the diffusion MRI tracking methods used in this work; improvements in spatial resolution and in the definition of cortical regions may lead to improvements in the percentage of accurately identified connections and nonconnections. In this study, we did not compensate for variations in the sizes of the cortical regions, that is, more streamlines will propagate from larger cortical regions such as V1 than from smaller regions. While this will reflect the true underlying anatomy, tracer injections tend to be comparable in absolute size and are typically not scaled to area size, so this would introduce some differences between tractography and tracer-derived results.

We investigated the performance of variables such as distance-based corrections, curvature, and FA constraints, which are commonly used when performing tractography, against in vivo tracer results, allowing a better understanding of their true effects. Without distance correction, the TP and FP rates both decline with increasing distance. Both the R and R²-corrections show improvements in the TP rate at long distance, but the FP rate is also increased, resulting in little gain in overall accuracy. Therefore, a more sophisticated correction method is needed if distance effects are to be compensated for.

Unexpectedly, we found that use of the FA thresholds tested reduced the accuracy of the connections found. This is possibly due to the rejection of streamlines that are actually TP pathways. This may be confounded by the necessity to segment gray matter to avoid the application of FA thresholds in these regions. If segmentation is not accurate, or if partial volume problems lead to regions of low FA beyond the identified cortical boundaries, this could contribute to the rejection of TP pathways emerging from the gray matter or entering the gray matter at the terminus of streamlines. In situations where the aim of tractography is not to establish connections between gray matter regions per se, but perhaps to segment a specific white matter pathway without its gray matter entry points, then it may still be possible that FA
thresholds could be helpful; our methodology is not able to answer this point definitively.

Although theoretically a curvature threshold appears to be very useful in excluding streamlines that are anatomically doubtful, it may be that such sharp changes in the direction of the streamlines have a minimal effect on the outcome of probabilistic tractography, unlike in deterministic tractography, where a single erroneous change of direction could have severe consequences. This could explain why such a constraint has little or no effect on our results. The slight variation in the results obtained on repeating the experiments using different curvature thresholds may simply be due to the Monte Carlo sampling that is used by PICo, which will introduce some variability. Arguably, the nature of probabilistic tractography dictates that all possible fiber orientations at a given point along a pathway are valid, with the probability of each being chosen determined by the

Figure 5. Effect of distance correction, $R$-correction (A–D) and $R^2$-correction (E–H) on: (A and E) the average ROC for SCI matrices at a range of acceptance thresholds in comparison with FVE91. (B and F) Accuracy of connections from the SCI matrices at a range of acceptance thresholds from 1% to 40%, compared with FVE91. (C and G) Percentage of TN and (D and H) % of TP connections identified when optimizing the SCI matrices generated for D1 and D2 against the Felleman and Van Essen atlas.

Note that results are only shown for acceptance thresholds between 1 and 40%, although the full range of threshold levels up to 100% was tested.

Figure 6. (A) TP rates and (B) FP rates when using no correction, $R$-correction, and $R^2$-correction, as a function of distance away from the ROI seed point averaged across D1 and D2.
intravoxel PDF alone; additional orientational thresholds are therefore applying post hoc cutoffs on the PDFs, which is at odds with the probabilistic framework of the technique. The lack of influence of curvature constraints in our results may simply be indicating that the bootstrap generation of the PDFs is sufficient to guard against a high probability of pathways with high curvature. It is possible that if our default step size was larger, curvature constraints may have been more important.

Our results are comparable with those of Thomas et al. (2014), showing similar performance on the ROC curves (e.g., compare results in Fig. 3 with the CSD results in Fig. 2 of the work by Thomas) despite consideration of different brain regions. While Thomas interpreted these results in a negative light, we are more optimistic as we believe these measures represent the lower limits of accuracy due to imperfections in the results of the tracer studies. However, we agree that tractography is fundamentally limited in its ability to detect long-range anatomical projections.

**Conclusion**

Our results demonstrate that tractography can identify the majority of expected anatomical connections in the visual network of the macaque brain and provide useful data to help define the limitations of the method. However, some caution is needed in interpretation of these results as it is falsely assumed that the invasive tracer studies provide a “gold standard” measure of connections. This limitation may be apparent in our data, where certain connections were present in both MR diffusion imaging datasets, but were absent in the Felleman and Van Essen atlas (a limitation that is partly confirmed by more recent invasive tracking data). Our results therefore represent a lower boundary on the true accuracy of connection identification using tractography. One further limitation of the current study is that it focuses exclusively on identifying the presence or absence of interareal connections, whereas actual connection strengths vary by many orders of magnitude. This comparative approach could prove useful in future studies aiming to test the performance of different tractography algorithms, or to try and identify the optimum acquisition and postprocessing parameters.

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**Notes**

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