7

Applications

THE AIM of neighbourhood tractography is to facilitate comparative analysis between subject groups in clinical studies, in a tract-specific manner. The brains of unhealthy or agèd individuals are, however, often substantially different from those of healthy young volunteer subjects. It is therefore important to confirm that the topological tract matching principle by which NT works remains valid in these cases. This chapter describes the application of NT to the clinical study of normal ageing and schizophrenia, and demonstrates that gains in segmentation consistency can be obtained even when the reference tract is drawn from a different population to the candidate tracts. The work described here was completed collaboratively with Jakub Piątkowski and Dr Susana Muñoz Maniega.

7.1 Tractography in the ageing brain

We discussed in §4.5 that normal ageing is a significant area of clinical interest in which DMRI has already begun to make a useful contribution. Early DTI studies of the effects of ageing on white matter, such as those by Pfefferbaum *et al.* (2000) and O'Sullivan *et al.* (2001), used manual segmentation of large white matter regions of interest, and demonstrated negative correlations between diffusion anisotropy and age. O'Sullivan *et al.* found a particularly strong effect in anterior white matter—a finding which has since been reproduced by Head *et al.* (2004); and for the corpus callosum genu in particular, by Abe *et al.* (2002). Kochunov *et al.* (2007) have additionally shown, using the TBSS technique, that FA in the genu shows a more robust association with other indices of structural health in the brain—such as average grey matter thickness—than does anisotropy in other white matter regions. Evidence for similar frontal effects in ageing monkeys has also been recently demonstrated (Makris *et al.*, 2007).

Considering this increasing body of evidence that suggests that DMRI-based indices such as FA may be useful for studying ageing, it is surprising that studies employing tractography-based

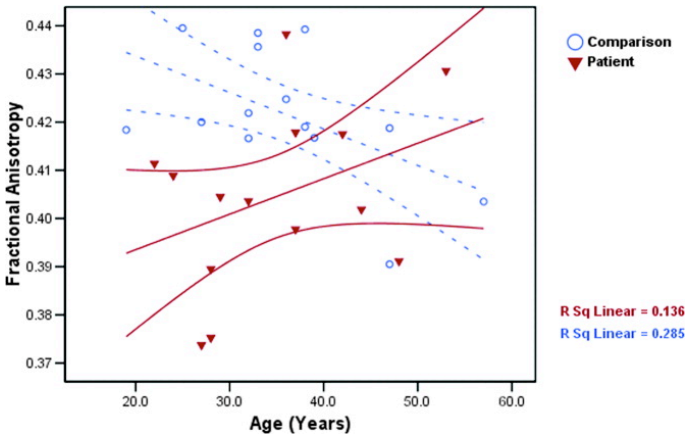

Figure 7.1: Relationships between age and FA in schizophrenics (red triangles) and healthy controls (blue circles). Points are averages over eight tracts. R^2 values for linear fits are given in each case. Reproduced from Jones *et al.* (2006).

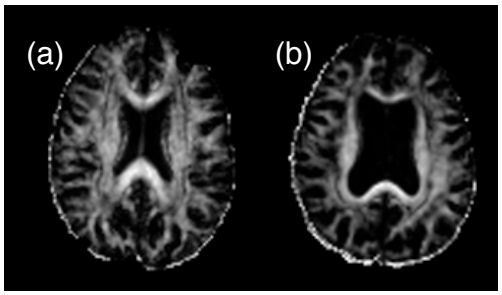

Figure 7.2: The brain of a young adult **(a)** differs most obviously from that of a healthy elderly subject **(b)** in the volume of the ventricles. This difference is clearly visible in these AVF maps.

segmentation for examining specific tracts appear to be almost nonexistent. Such tract-specific information, obtained in a more objective manner than is possible with manual segmentation, could be particularly helpful for confirming or contradicting the suggestion that frontal white matter decline is particularly marked during normal ageing. Jones *et al.* (2006) provided evidence that the relationship between anisotropy and age appears to be different in schizophrenic patients to controls in general (see Fig. 7.1), but their tract-specific measurements relate only to the effects of schizophrenia, and are therefore not especially helpful in understanding the impact of ageing in the healthy population.

Tractography in the agèd brain encounters additional challenges, compared to similar tracking in young adults. Firstly, since anisotropy is generally lower, the level of uncertainty associated with dMRI estimates of fibre orientation can be expected to be higher. This may make consistent segmentation of particular tracts intrinsically more difficult. Secondly, the morphology of older brains usually differs from younger ones—in particular, grey matter volume tends to shrink and the CSF-filled ventricles become larger (see Fig. 7.2). If this effect turns out to be highly variable among a population of agèd brains, then using a reference tract to guide tract matching may not be as reliable as in younger brains.

To test the performance of NT in an agèd population, 27 healthy volunteers aged over 65 were subjected to a dMRI protocol using a single-shot spin-echo echo-planar imaging sequence with 64 noncollinear diffusion weighting gradient directions at a b-value of 1000 s mm^{-2}, and 7 T_2-weighted scans. 53 contiguous axial slice locations were imaged, with a field of view of 240×240 mm, and a slice thickness of 2.5 mm. The acquisition matrix was 96×96 voxels in-plane, zero filled to 128×128. TR was 13.5 s per volume and TE was 75 ms. It should be noted that these parameters differ a little from those used for the study described in chapter 6, although all subsequent image preprocessing steps were the same.

These data were acquired as part of a study called DELCORT, whose principal investigator is Dr Alasdair MacLullich, a Lecturer in Geriatric Medicine at the University of Edinburgh. All image preprocessing, tractography and reference tract selection for this section was carried out by Jakub Piątkowski, with assistance from Dr Mark Bastin and the author.

The fasciculi of interest that were used for testing NT in this agèd cohort were the genu and splenium of the corpus callosum; the corticospinal tract (CST, left and right)[a]; and the cingulum bundle (CB, left and right). The registration method for seed point placement was used to transfer a single point for each tract from MNI standard space (Evans *et al.*, 1993) to each individual's brain. A reference tract was then selected by hand from the set of native space tracts, whenever an acceptable segmentation was available. In the corpus callosum genu, however, none of the tracts generated in this way was satisfactory, and so a seed point was hand selected in a single subject's brain volume to give a good match—which was then used as the reference tract—and the seed was transferred to standard space using the inverse of the usual transformation. The resulting set of six reference tracts are illustrated in Fig. 7.3. The genu and splenium tracts were drawn from a single subject, the two cingulum bundles from another subject, and the two CSTS from two more subjects.

NT was applied in each remaining subject for each fasciculus, using a neighbourhood size of $7 \times 7 \times 7$ voxels. Tracts segmented using the registration method and NT were inspected by eye to establish whether or not they were anatomically plausible representations of the relevant

[a]The corticospinal tract is the fasciculus that was segmented by seeding in the posterior limb of the internal capsule in chapter 6.

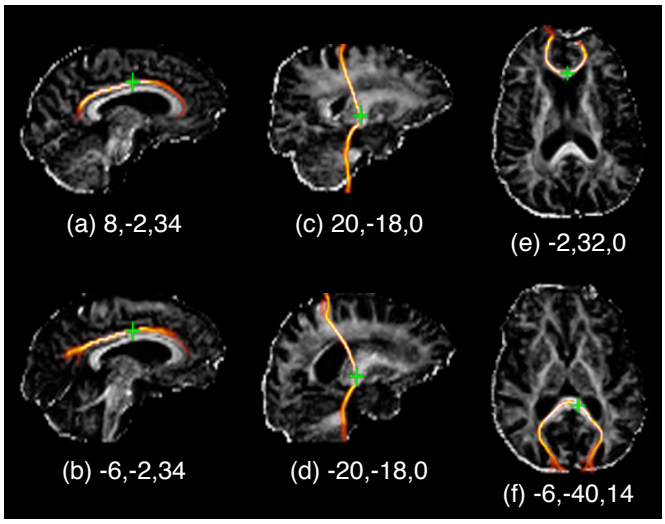

Figure 7.3: Reference tracts used for the ageing study, representing right **(a)** and left **(b)** cingulum bundle, right **(c)** and left **(d)** corticospinal tract, genu **(e)** and splenium **(f)**. Coordinates of the original seed points in MNI space are given in each case, and native space seeds are marked with green crosses. Images courtesy of Jakub Piątkowski.

fasciculus in each case. Finally, using the field of connection probabilities associated with the selected candidate tract, $\phi(\mathbf{x})$, as a set of voxel weightings, tract-averaged values of AVF, FA and MD were calculated according to

$$F = \frac{\sum_{\mathbf{x}} \phi(\mathbf{x}) f(\mathbf{x})}{\sum_{\mathbf{x}} \phi(\mathbf{x})}, \tag{7.1}$$

where $f(\mathbf{x})$ is a scalar field encapsulating the values of AVF (and so on) at each voxel in the brain. Since we are hoping to make group contrasts more robust for comparative studies, we would hope that the variability of these measures would be smaller within this group using NT than with the registration method.

Table 7.1 shows the subjective results of examining each tract by eye to determine whether or not it represents an anatomically plausible segmentation of the relevant fasciculus. The table also shows the percentages of tracts whose segmentations were deemed better or worse using neighbourhood tractography, irrespective of whether or not the NT segmentation was actually good enough to be considered acceptable. Table 7.2 shows the coefficients of variation (CVs) for each metric, calculated using Eq. (7.1) from the single tracts selected with registration or NT. All selected tracts contributed to these values, whether or not they were found to represent acceptable segmentations.

The subjective and objective results largely corroborate one another. Coefficients of variation for each of the three DMRI metrics are generally lower using NT than they are with the registration method, except in the right corticospinal tract—which was also the only tract in which NT was judged to have worsened more tract segmentations than it improved. The CVs for MD in the left cingulum and left CST were also higher using neighbourhood tractography, but the differences in these cases were so small as to be negligible. It is clear, however, that

%	Right CB	Left CB	Right CST	Left CST	Genu	Splenium	Total
RM acceptable	18.5	18.5	22.2	37.0	51.9	3.7	25.3
NT acceptable	44.4	48.1	14.8	40.7	81.5	74.1	50.6
Either acc.	48.1	59.3	29.6	59.3	100.0	74.1	61.7
Neither acc.	51.9	40.7	70.4	40.7	0.0	25.9	38.3
NT better	59.3	55.6	11.1	37.0	48.1	81.5	48.8
NT worse	11.1	14.8	48.1	22.2	18.5	0.0	19.1

Table 7.1: Proportions of tracts generated by applying the registration method (RM) or neighbourhood tractography (NT) to the agèd cohort which are considered "acceptable" matches, expressed as percentages. Proportions of tracts which were deemed better or worse matches after applying NT are also given. The reference tracts are included in this analysis.

Metric	Method	Right CB	Left CB	Right CST	Left CST	Genu	Splenium
AVF	RM	0.274	0.328	0.080	0.098	0.362	0.256
	NT	0.165	0.260	0.117	0.086	0.167	0.198
	difference	0.109	0.068	−0.037	0.012	0.195	0.058
FA	RM	0.242	0.298	0.075	0.095	0.282	0.159
	NT	0.152	0.229	0.113	0.083	0.141	0.136
	difference	0.090	0.069	−0.038	0.012	0.141	0.023
MD	RM	0.296	0.484	0.053	0.042	0.293	0.296
	NT	0.073	0.489	0.059	0.049	0.105	0.204
	difference	0.223	−0.005	−0.006	−0.007	0.188	0.092

Table 7.2: Coefficients of variation for each metric and fasciculus, across the agèd cohort, using the registration method (RM) and neighbourhood tractography (NT). Differences are positive where the CV is greater using the registration method.

Metric	Method	Agèd mean	Young mean	p-value
AVF	RM	0.341	0.362	0.238
	NT	0.336	0.402	**0.002***
FA	RM	0.429	0.447	0.359
	NT	0.427	0.492	**0.006***
MD	RM	8.99	8.52	0.275
($\times 10^{-4}$)	NT	9.16	8.42	0.053
				$^*p < 0.01$

Table 7.3: Comparisons of the three tract metrics for the corpus callosum genu, between the agèd and young groups, using only visually acceptable segmentations. p-values were calculated using two-tailed t-tests.

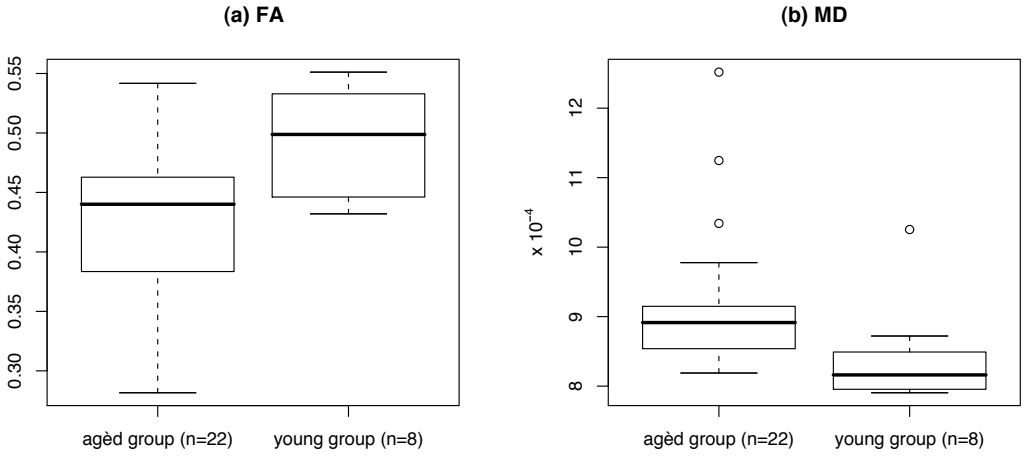

Figure 7.4: Box-and-whisker plots of weighted mean FA and MD in the genu of the agèd and young subject groups. The thick horizontal lines represent the medians for each group.

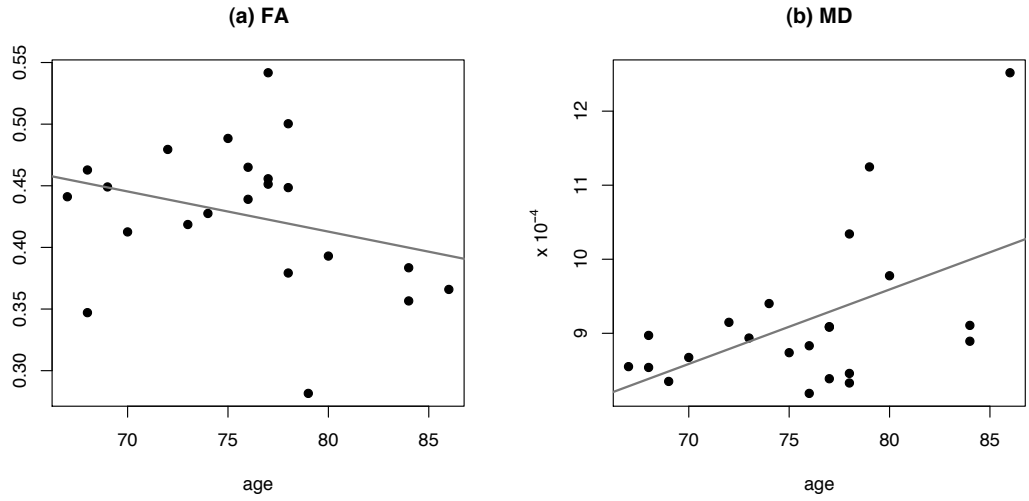

Figure 7.5: Scatter plots of age against genu FA and MD within the agèd subject group. Least-squares linear regression lines are shown in grey for information, but there is no significant correlation.

there was considerable variation among the fasciculi in the proportions of tracts found to be acceptable, and in the variability of tract metrics as indicated by the CV values. There are even substantial differences between bilateral pairs of tracts: twice as many tracts representing the left CST were successfully segmented using either of the two methods, for example, as for the right CST. This lack of consistency between comparable tracts across the data set may be a genuine characteristic of the data, but it is more likely that differences in reference tract quality are the main source of the effect. This is an issue that we will return to later.

7.2 Old versus young

We have described the effects of neighbourhood tractography in reducing the variability of diffusion metrics within a single population, but we have yet to demonstrate that this is helpful in performing group contrasts. To this end, the genu reference tract used for the study described above was used to perform NT in a group of eight young adults (mean age 25.8 ± 3.7 years), using the same neighbourhood size of $7 \times 7 \times 7$ voxels. The acquisition protocol for these subjects was described in §6.4. The three metrics of interest were calculated for the tract selected as the best match by NT, according to Eq. (7.1). These were then compared with the data from the 22 agèd subjects (mean age 75.7 ± 5.3 years) whose genu segmentations using NT were considered acceptable. Equivalent values were also computed using the registration method.

The results are tabulated in Table 7.3, and illustrated graphically in Fig. 7.4. We observe that the mean FA and AVF is significantly different between the groups using NT, according to a standard two-tailed t-test; but not with the registration method. In line with the results of Abe *et al.* (2002) and others, anisotropy is found to be higher in the younger group. The difference between MD means also approaches significance using NT, with $p = 0.053$. It therefore appears that NT does help with this type of contrastive analysis in specific tracts.

The box-and-whisker plots additionally give a sense of the variance in each group. We note that for both FA and MD[b] the interquartile range is similar for the two groups, but the full data range is considerably wider for the agèd group. The greater age variation *within* the agèd group is a plausible cause of these longer-tailed distributions, but further analysis did not

[b]The equivalent plot for AVF (not shown) closely resembles the FA plot, since the two measures are closely related.

reveal any significant age effect (see Fig. 7.5). Hence we can only conclude that there are some uncontrolled covarying factors which differ more within the older group than the younger one.

7.3 Improving the reference tracts

Although we have demonstrated in the previous section that a reference tract can be successfully used for segmenting tracts in multiple data sets acquired with different dMRI protocols, it must be admitted that the "hit rate", as indicated by the proportions of tracts deemed acceptable in Table 7.1, is not especially high in the agèd cohort. This will be partly due to lower data quality in this group: the reduced anisotropy will mean that orientational uncertainty is higher, and so tracking will be less reliable and more prone to diverge from the expected trajectory. Another factor is anatomical differences between subjects such as variation in ventricle size, which may tend to make the placement of the neighbourhood in native space inappropriate in some cases. A possible remedy for this is to increase the neighbourhood width. The reference tract itself, however, is an extremely important aspect of the neighbourhood tractography process; and by selecting a tract more or less arbitrarily from the data set under study we are neglecting to ensure the quality of the reference, either as a typical example of the fasciculus it represents or in terms of its optimality for the neighbourhood tractography algorithm.

In the following work, which was conducted jointly with Dr Susana Muñoz Maniega, we describe a method for defining reference tracts based on a published human white matter atlas (Mori *et al.*, 2005). These references aim to be both independent of any particular data set and carefully constructed so as to minimise ambiguity for the tract matching algorithm. This will hopefully maximise the transferability of the reference tracts, which is a major benefit of the general NT approach. In the following section we apply these references to data from the Edinburgh High Risk study (principal investigator Prof. Eve Johnstone), which involves schizophrenics and relatives considered to be at high risk of becoming schizophrenic themselves.

We begin by explaining our motivation more explicitly. The aim of a reference tract is to epitomise the topological characteristics of the fasciculus which we wish to segment in an individual brain volume. Naturally, the shape and length of the correct segmentation in any given subject's brain will not be identical to those of any reference tract, but the tract similarity metric that we described in chapter 6 is designed to allow us to maximise the correspondence, given the constraints imposed by the data. There is, in effect, a distribution over tract topologies, from which the fasciculus of each individual is drawn. In order to maximise the effectiveness of the neighbourhood tractography method, the reference tract should represent a topology that is as close as possible to the mode of this distribution; thus ensuring that the greatest possible proportion of "correct" segmentations are considered good matches to it. A reference tract chosen from a single subject may in fact sit within the tails of the distribution—i.e. it may be an atypical outlier—even if it is appropriate for that subject, and appears to be plausible. To create a separate reference tract for each data set would also involve an undesirable and unnecessary increase in the work required to apply NT to new studies. On the other hand, atlas representations of white matter tracts are typically based on data from several subjects, and therefore give a sense of the underlying distribution.

With reference to the white matter atlas created by Mori *et al.* (2005), we manually segmented, in the MNI single subject template brain (Holmes *et al.*, 1998), the whole region corresponding to the tract of interest. We then resampled this region to correspond to the resolution of the native space in which the data for the High Risk study were acquired. Note that only a scale transformation is applied here, so this resampling process is quite subject-independent.

An example of the tract region at this stage, overlaid on an appropriately resampled image of the MNI single subject, is shown in Fig. 7.6(a). The tract in this case is the right uncinate fasciculus. This region represents all voxels in the brain through which the tract may pass, but it is considerably wider than any single tract would be. It is therefore unrepresentative, and it is also heavily suboptimal for the NT similarity algorithm, because there is no unique maximum intensity pathway through it. Our final aim is a very narrow pathway running through the centre of this region, which should be a good approximation to the mode of the

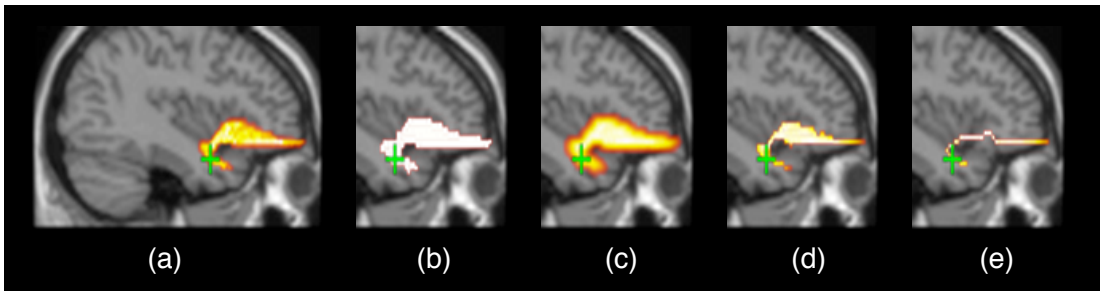

Figure 7.6: The steps of atlas-based reference tract generation, demonstrated on the right uncinate fasciculus. Each image is shown as a sagittal maximum intensity projection, overlaid on the slice of the MNI single subject template in-plane with the seed point. The seed is shown in green.

spatial distribution over tracts, and unambiguous for the purposes of matching. We achieve this by first binarising the image, giving all nonzero voxels the same value (b); smoothing with a Gaussian kernel with standard deviation of 2 mm, thereby encoding at each voxel the distance to edge of the region (c); and then skeletonising the result using the same principle that the TBSS technique uses for skeletonising FA maps (d). The latter skeletonisation process works by finding local maxima in image intensity (cf. §6.1). What remains is a "core" of the original region, from which a reduced tract is calculated (cf. §6.3.1), producing the final reference tract (e), which has single voxel thickness along its length and is therefore unambiguous in orientation at each step of the matching algorithm. Seed points for these reference tracts are placed to avoid regions where fibres are expected to cross, or where contaminating tract orientation information might otherwise be expected to be present.

7.4 A schizophrenia study

Evidence from functional imaging has led to the suggestion that schizophrenia may be a disconnection syndrome, in which interaction between frontal and temporal regions is particularly abnormal (Friston & Frith, 1995). As a result, there is a considerable literature of white matter studies in schizophrenia, and DMRI methods are now commonly applied as part of them. Voxel-based analyses have provided evidence of DMRI-visible changes in the uncinate and arcuate fasiculi (Burns *et al.*, 2003) and cingulum bundle (Kubicki *et al.*, 2003), amongst other regions. Park *et al.* (2004) also demonstrated consistent hemispheric asymmetries in the anisotropy of a number of white matter structures, in both healthy and schizophrenic subjects.

The use of tractography in studies of schizophrenia has so far been limited. Kanaan *et al.* (2006) and Price *et al.* (2007) both use tractography methods to demonstrate reduced corpus callosum FA in schizophrenics, while Jones *et al.* (2006) examine a number of tracts but find a significant difference in FA only in the left superior longitudinal fasciculus.[c]

In preparation for this study, reference tracts for the two cingulum bundles, arcuate fasciculi (AF), uncinate fasciculi (UF) and anterior thalamic radiations (ATR) were created as described above. The latter fasciculus connects prefrontal cortex to the thalamus through the anterior limb of the internal capsule—its pertinence is due to evidence of reduced anterior thalamic grey matter density in schizophrenia (McIntosh *et al.*, 2004), which might be linked to a breakdown of connectivity between this part of thalamus and cortex.

27 schizophrenic patients (mean age 36.5 ± 9.2 years), 20 healthy subjects at an enhanced risk of becoming schizophrenic due to having relatives with the disorder (mean age 30.2 ± 2.9 years) and 50 healthy controls (mean age 35.3 ± 10.9 years) underwent the DMRI protocol described in §6.4. These data were acquired by Dr Dominic Marjoram and Dr Andrew McIntosh. For each of the eight tracts of interest, neighbourhood tractography was applied to each subject, using a

[c]The superior longitudinal fasciculus and arcuate fasciculus are closely related structures, and the names are often used interchangeably; although recent work suggests that they should not be considered identical (Makris *et al.*, 2005). It is not clear what definition of the fasciculus is being used by Jones *et al.* in this context.

Tract	% acceptable	Control mean FA	HR mean FA	Schiz. mean FA	p-value
Right CB	73.2	0.407	0.384	0.406	0.407
Left CB	83.5	0.430	0.413	0.390	0.111
Right AF	70.1	0.418	0.423	0.406	0.395
Left AF	83.5	0.444	0.419	0.437	**0.028***
Right UF	94.8	0.364	0.354	0.347	0.258
Left UF	92.8	0.380	0.357	0.357	0.059
Right ATR	71.1	0.355	0.347	0.342	0.719
Left ATR	77.3	0.389	0.367	0.362	0.096
					$^*p < 0.05$

Table 7.4: Bilateral results based on weighted FA values calculated in the cingulum bundles (CB), arcuate fasciculi (AF), uncinate fasciculi (UF) and anterior thalamic radiations (ATR). Group means were calculated for control, high risk (HR) and schizophrenic subjects and compared using a one way ANOVA. p-values given are derived from a standard F-test.

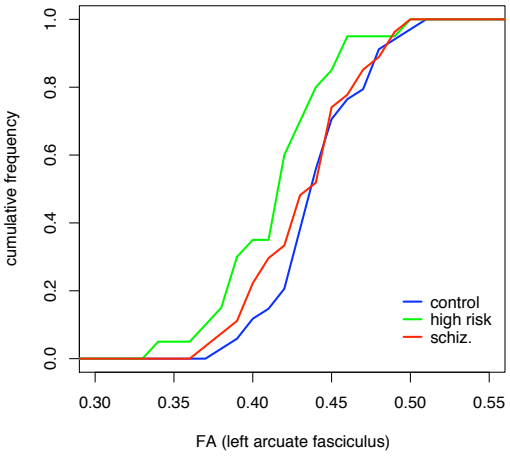

Figure 7.7: Cumulative frequency plots of weighted mean FA in the left arcuate fasciculus of each group. Bonferroni corrected t-tests found a significant difference between high risk subjects and controls only.

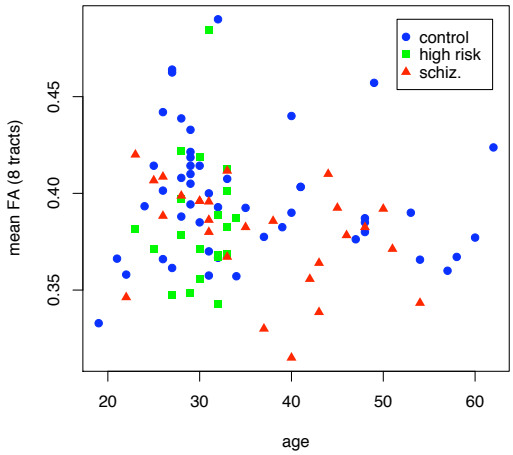

Figure 7.8: Scatter plot of age against mean FA across all of the tracts used in this study.

neighbourhood width of $7\times7\times7$ voxels as before. The proportions of visually plausible tracts were recorded in each case, and for these acceptable segmentations, a weighted mean FA value was calculated as per Eq. (7.1). For each tract, a one way analysis of variance (ANOVA) was applied to establish whether there was any effect of group membership on anisotropy. We also examined the relationship between age and anisotropy, averaged over all tracts, for each group individually.

ANOVA results are given in Table 7.4. We observe that mean FA in controls is higher than the other two groups in seven of the eight tracts—the right arcuate fasciculus is the only exception—but there is a significant effect of group membership only in the left arcuate. This result is consistent with the findings of Jones *et al.* (2006), but post-hoc *t*-tests applied to these data showed that the significant difference was between controls and the high risk group, with a Bonferroni corrected *p*-value of 0.036. The mean in the schizophrenic population was considerably higher than the high risk mean for this tract. The lack of significance between schizophrenics and controls might be related to greater variance in the former population, but a cumulative frequency plot (Fig. 7.7) does not bear this hypothesis out. The general steepness of the curve—which hints at the spread of the data—is similar between the control and schizophrenic populations. There is perhaps a tendency for FA values below the group median to be lower in schizophrenics than controls, but in general the two curves are genuinely very similar. The high risk curve, by contrast, is consistently shifted towards lower FA values.

We additionally note, in common with Park *et al.* (2004), that there is a noticeable lateralisation effect in mean FAs, which are invariably higher, on average, in the left hemispheric versions of each tract.

Fig. 7.8 shows a scatter plot of age against the average FA across all tracts. Our set of eight tracts was not identical to those used by Jones *et al.* (2006), but we nevertheless failed to find evidence of the general age effect described in that study. Of the three groups, only the schizophrenics yielded a statistically significant relationship (Spearman's $\rho = -0.43$, $p = 0.024$), but the correlation was negative in this case, not positive as in Fig. 7.1.

There are any number of reasons that might help to explain why relationships between clinical status and tract FA were not more numerous. There may be genuinely little effect on white matter; FA may not be sensitive to the kinds of physiological abnormality associated with schizophrenia, or only inconsistently so; or the effect may be so small that it is masked by noise. Jones *et al.* (2006) suggest that the age of onset of schizophrenic symptoms may be a relevant covarying factor to include in a more complex analysis. Since there was no difference between controls and schizophrenics in the left arcuate fasciculus, it is difficult to interpret the finding of difference between the control and high risk groups. Because the latter was not very strongly significant, it may be simply coincidental.

Despite a paucity of clinical findings under the relatively simple analysis that we have applied here, the considerably higher acceptance rates—reaching up to 95%—for tracts segmented using atlas-based reference tracts are encouraging. Of course, it would be necessary to use these tracts in the agèd cohort in order to make a direct comparison between the two types of reference—the subjects involved in this study are, after all, noticeably younger. However, even if the improvement is robust, it is not yet large enough to allow us to dispense with manual checking of the selected tracts; and in small subject groups the rejection rate may still be considered unacceptably high. The limitations of the similarity measure discussed in §6.6 remain in any case. There is certainly room for improvement in the neighbourhood tractography method itself.

7.5 Conclusions

Despite some evidence of greater variability among the older volunteer population that we studied in the first half of this chapter, compared to the younger volunteer group, we have found that a reference tract drawn from one group can be used to successfully guide the selection of candidate tracts in the other. However, the proportions of tracts successfully segmented using a reference drawn from the data set, as estimated by a human observer, was somewhat lower than might be hoped. A marked improvement was found using reference

tracts based on a white matter atlas—although different raters were involved in these two studies, so some of the difference may be attributable to inconsistency in acceptance criteria.

The discussion of intersubject distributions over tracts in this chapter raises the possibility of using a formal probabilistic model to represent this variability. With proof of concept for neighbourhood tractography in place, refinement of the method is our next priority.