Diffusion MRI parameters of corpus callosum and corticospinal tract in neonates: comparison between region-of-interest and whole tract averaged measurements

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1. Introduction

Structural and diffusion magnetic resonance imaging of the brain during the newborn period contributes to understanding the neural systems that underpin typical and atypical development. Preterm birth is closely associated with injury and / or maldevelopment of cerebral white matter[1], and with several long-term adverse outcomes including cerebral palsy, neurocognitive impairment, social difficulties and vulnerability to psychiatric disease[2-5].

Quantitative parameters derived from diffusion magnetic resonance imaging (dMRI) include fractional anisotropy (FA) and mean diffusivity (MD), which enable inference about the microstructural organisation of brain tissue and white matter tracts[6]. A consistent finding is that FA is decreased and MD is increased in the white matter of preterm infants at term equivalent age[7-10]. The corpus callosum (CC) and corticospinal tracts (CST) are of particular interest in the context of developing biomarkers for later neurodevelopmental outcome after preterm birth, because dMRI parameters in these tracts are influenced by gestational age, and their microstructural properties have been associated with later function[11-13].

Abbreviations

CST, corticospinal tract; GA, gestational age; gCC, Genu of corpus callosum; ICC, Intraclass correlation; OFC, Occipital-frontal head circumference; PLIC, posterior limb of internal capsule; PNT, probabilistic neighbourhood tractography

Two widely used methods to measure dMRI parameters in these tracts include region-of-interest (ROI) analysis and tractography, but the agreement of values obtained using these methods is uncertain; for example, ROI measures show an inconsistent correlation with values of the white matter skeleton at corresponding sites using tract-based spatial statistics[14]. ROI approaches are often used in clinical settings because they provide absolute quantification of MD and FA in selected regions without the need for substantial post-processing but they are labour intensive, prone to operator bias, and the volume and shape of the ROI can influence results[15-17].

Probabilistic Neighbourhood Tractography (PNT) is an automatic segmentation method, which provides whole tract-averaged measures of FA and MD in major white matter fasciculi. This method, first described by Clayden et al[18,19], has been optimized for neonatal data[20]. It involves single seed point tractography to segment a tract of interest by modelling the length and shape variation of individual tracts compared with a pre-defined reference tract.

We aimed to compare FA and MD values measured from geometric ROIs with tract-averaged values obtained using PNT in the genu of the corpus callosum (gCC) and the CST. The secondary aim was to evaluate intra-rater variation in FA and MD values obtained by ROI placement within these tracts.

2. Materials and methods

2.1 Participants

Ethical approval was granted from the National Research Ethics Service (South East Scotland Research Ethics Committee) and informed parental consent was obtained. The study was conducted in accordance with the 18th World Medical Assembly, Helsinki 1964 and later revisions. Infants were recruited from the Neonatal Intensive Care Unit and postnatal wards between June 2012 and June 2014. The group consists of a subset of infants recruited to a longitudinal study of the effect of preterm birth on the developing brain and neurodevelopmental outcome. Preterm infants (birth weight <1500 grams or gestational age <32 completed weeks) and term infants (birth after 37 weeks gestation) were recruited. All infants underwent MRI at term equivalent age (38 to 42 weeks' gestational age). Infants with chromosomal or congenital abnormalities and those with major parenchymal lesions including haemorrhagic parenchymal infarction and cystic periventricular leucomalacia were excluded.

2.2 Magnetic Resonance Imaging

A Siemens Magnetom Verio 3 T MRI clinical scanner (Siemens Healthcare GMbH, Erlangen, Germany) and 12-channel phased-array head coil were used to acquire: T1-weighted MPRAGE volume (~1 mm^3 resolution), T2-weighted STIR (~0.9 mm^3 resolution), T2-weighted FLAIR (~1 mm^3 resolution), and diffusion MRI (11 T2- and 64 diffusion encoding direction (b = 750 s/mm^2) single-shot spin-echo echo planar imaging (EPI) volumes with 2 mm isotropic voxels) data.

Infants were examined in natural sleep with pulse oximetry, temperature and electrocardiography data monitoring. Ear protection was used for each infant, comprising earplugs placed in the external ear and neonatal earmuffs (MiniMuffs, Natus Medical Inc., CA).

2.3 Diffusion MRI Data Processing

After conversion from DICOM to NIfTI-1 format, dMRI data were pre-processed using FSL tools (FMRIB, Oxford, UK; http://www.fmrib.ox.ac.uk). This included brain extraction and removal of bulk infant motion and eddy current induced artifacts by registering subsequent diffusion-weighted volumes to the first T2-weighted EPI volume for each subject. Using DTIFIT, MD and FA volumes were generated for every subject. Underlying connectivity data was provided by FSL's BedpostX/ProbTrackX algorithm run with its default parameters: 2-fiber model per voxel, 5000 probabilistic streamlines for each tract with a fixed separation distance of 0.5 mm between successive points[21,22].

For ROI analysis, values were extracted from a 8 mm^3 cubic ROI placed at the centre of the genu of corpus callosum (manual visual placement in axial plane being midline between the superior margins of the lateral ventricles, below the cerebral cortex; Figure 1c), and right and left PLIC (manual visual placement in axial plane being inferior-lateral on the PLIC, seen as 2 diagonal bands of increased signal intensity within the basal ganglia; Figure 1f) on the T2-weighted EPI volume. 'In-house' MATLAB (http://uk.mathworks.com) code was written to allow the user to interactively place these cubic ROIs using a

mouse-driven GUI and generate FA and MD values from them; FA and MD parametric volumes are, by definition, co-registered to the T2-weighted volume for each subject.

For PNT analysis, we used the method first described by Clayden et al[18,19] and refined by Anblagan et al[20] for the neonatal brain. Reference tracts for the gCC (Figure 1a) and CST (Figure 1d, e) were generated from a previous study of 20 term-control infants[20]. Individual tracts of interest (gCC, right and left CST) were segmented using PNT as implemented in TractoR (http://www.tractor-mri.org.uk). The resulting tractography mask was applied to each subject's MD and FA maps and tract-averaged values were calculated (see Figure 1b for an example segmentation of the genu in a representative subject, and Figure 2a-c for examples of segmented CC and CST in 3D space).

2.4 Statistical analysis

Pearson correlation was used to investigate the strength and direction of linear relationship between both FA and MD derived from ROI and PNT for each tract; correlation coefficient (*r*) values of 0.00-0.39, 0.40-0.59, and >0.6 were defined as weak, moderate and strong respectively, and $p<0.05$ was considered statistically significant. Bland-Altman statistics were used to investigate agreement between FA and MD values derived from ROI and tract-averaged approaches for each tract; the mean difference (95% limits of agreement) is reported. Intraclass correlation coefficient (ICC) was used to estimate intra-rater variability by a single observer who repeated ROI

procedures on two occasions (S.A.S.) for each tract; strength of correlation was classified as weak, moderate and strong using the thresholds stated above. Statistical analyses were carried out using SPSS v21 (SPSS Inc., Chicago, USA).

3. Results

3.1 Participants

The study group consisted of 81 neonates (41 female, 40 male) of which 15 were born at term. Participants underwent MRI at term equivalent age, and characteristics are shown in Table 1.

3.2 Tract-averaged versus region-of-interest measurements for genu of corpus callosum and corticospinal tracts

FA and MD values generated from PNT and ROI placement in gCC and left and right CST are shown in Table 2. For each tract, FA and MD derived from both methods had weak to moderate correlation (Pearson coefficient [*r*] 0.24-0.54, all p-values < 0.05).

The magnitude of difference in FA measured using PNT or ROI ranged between 0.13-0.17 for the 3 tracts, and none had 95% limits of agreement that included the possibility of no difference. Therefore, for FA, ROI measures extracted from the center of the gCC and the PLIC do not reflect whole tract-averaged values from the gCC or CST respectively (Figure 3a-c).

The magnitude of difference in MD measured from PNT and ROI ranged between 0.101 and 0.184 $\times 10^{-3}$ mm^2/s across the studied tracts. The mean difference in MD in gCC derived from PNT and ROI placement was low (0.101

$\times 10^{-3}$ mm^2/s) and included the possibility of no mean difference, but tract-averaged MD values for right and left CST disagreed with values obtained from ROI placement in the right and left PLIC respectively (mean difference 0.136 [95% limits 0.005-0.267], right; mean difference 0.184, [0.062-0.307], left), Figure 3d-f.

3.3 Intra-rater agreement for region of interest measurements

Intraclass correlation for intra-rater variation in ROI measurements of FA and MD was moderate to strong across all tracts (ICC 0.56-0.94, $p<0.001$ for all), Table 3. Intra-rater ROI measurements for FA and MD showed that the mean difference for each tract was marginal, with difference in FA values between 0.0005 and 0.004 and difference in MD values between 0.003 and 0.005 $\times 10^{-3}$ mm^2/s. The 95% limits of agreement for both FA and MD in all tracts included the possibility of no difference.

4. Discussion

The results of this study indicate that FA and MD values estimated from regions of interest within the genu of the corpus callosum (gCC) and the corticospinal tracts (CST) show weak correlation and low agreement with tract-averaged values. Since intra-rater variability in ROI measurement of FA and MD was low (mean ICC>0.75 for five out of six tracts), these observations do not appear to be substantially explained by operator bias in ROI placement. The study suggests that sampling ROIs within major fasciculi does not provide sufficient representation of white matter tract microstructure in the newborn. The magnitude of disagreement in FA and MD was large and this

has implications for research designed to evaluate neonatal image biomarker qualification and validation because relatively modest changes in FA and MD in the CC and CST in the newborn period are associated with neurodevelopmental outcome in childhood[9,11,23.] Therefore, the differences observed between dMRI parameters derived from ROI and PNT values may include clinically significant values.

The differences in dMRI parameters observed in ROIs versus tract-averaged values could be explained by within-tract variation in maturational processes that influence tissue microstructure. In early development, white matter tract myelination proceeds in phases: the first is characterized by proliferation and maturation of oligodendrocytes in contact with axons, an increase in cell membrane density and a reduction in brain water content; and the second involves ensheathment of oligodendroglial processes around axons[6]. Each phase is associated with changes in the molecular motion of water; specifically, changes in anisotropy precede the appearance of myelin[24-26]. Our data are consistent with the observation that these processes show considerable spatial variation within tracts. As the genu of the corpus callosum is unmyelinated in the neonatal period[27], the higher FA and lower MD values sampled from the ROI placement (Figure 1c) compared to tract averaged values could be attributable to microstructural differences within the tract that precede myelination. The CST is partially myelinated at the end of gestation[27] with MRI evidence of myelin in posterior limb of the internal capsule and tracts of the pre- and post- central gyri apparent by term equivalent age[28], although myelination of the whole tract is not complete until

the second year of life. The high FA and low MD observed in the PLIC ROI compared with whole CST could therefore represent variation of myelination within the tract, or it could be attributable to other properties of tract maturation that influence dMRI parameters described above.

We studied the CST because injury to the developing brain is strongly associated with motor impairment[3], and the development of early image markers of injury to motor systems could enable more effective stratification of high-risk groups for trials of early interventions. The CST is readily segmented using a range of neonatal tractography techniques[29-33]; and we observed comparable values of FA and MD to those reported by others, suggesting that the study group is representative. The posterior limb of the internal capsule is of particular interest because of its close association with motor outcome following different forms of perinatal brain injury[34]. The corpus callosum provides the neural substrate for transfer of interhemispheric information, and segmentations from neonatal MRI have shown that its macro- and micro-structure are influenced by perinatal events, and are linked to outcome[23, 32-33, 35-38]. The weak agreement we observed for FA and MD values derived from ROIs in CST and gCC compared with whole tract-averaged values from the respective tracts, suggests that ROI approaches do not represent microstructural characteristics of the whole tract unit, and that the reliability of neonatal DTI biomarkers is strongly influenced by site of measurement.

We found that intra-observer agreement of FA and MD values obtained by manual ROI placement in gCC and PLICs was high, which is consistent with

agreement studies reported in the literature[17] for these specific structures. However, ROI analysis is a subjective method and variation in measurements may be explained by slice choice and manual ROI placement. Angulation between slices may alter tract volume and ROI placement close to the edge of a tract may capture CSF or grey matter, which have different FA and MD values to white matter structures[39]. Therefore, ROI analysis requires an operator with experience in neonatal neuroanatomy to maximize the validity of results, and difficulties ensuring accurate placement of ROIs within white matter networks limits application of this approach.

The study has some limitations. First, we studied ROI versus tract-averaged agreement of FA and MD values in 3 major tracts because ROI placement was accurate and consistent for these tracts. Further work using high resolution DTI and rigorous protocols for consistent ROI placement would be required to determine whether ROI measurements achieve better representation of whole tracts in other neural systems. Second, we did not include specific myelin related imaging protocols such as magnetization transfer ratio[26, 40] or myelin water fraction[41], or methods for resolving multiple fiber orientations within voxels. In future work the use of these imaging techniques may be informative for understanding sources of within-tract variation in microstructure and, by inference, maturation observed in this study.

5. Conclusions

In conclusion, FA and MD values derived from regions of interest show weak agreement with tract-averaged values in the corpus callosum and corticospinal tracts. ROI approaches may not provide sufficient representation of whole tract microstructure in the developing brain. These observations have implications for the development of neonatal MR image biomarkers of perinatal brain injury and disease.

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Table 1. Clinical characteristics of participants

Characteristics	Patient Group (n=81)
Birthweight/ kg (range)	1.582 (0.55-4.26)
Mean gestational age at birth / weeks (range)	31.28 (23.28-41.71)
Mean gestational age at MRI scan / weeks (range)	40.28 (37.28-45.43)
Mean OFC at time of MRI scan / cm (range)	34.9 (31.0-38.0)
Mean weight at time of MRI scan / kg (range)	2.994 (2.06-4.26)
Vaginal delivery (%)	31 (38.3)
Histological evidence of chorioamnionitis (%)	19 (23.5)
Exposure to any antenatal steroids (%)	63 (77.8)
Exposure to antenatal magnesium sulphate (%)	30 (37.8)

Table 2. dMRI measurements generated by PNT and ROI methods in genu of the corpus callosum and the corticospinal tracts

	gCC		Right CST		Left CST	
	FA	MD $x10^{-3}$ mm^2/s	FA	MD $x10^{-3}$ mm^2/s	FA	MD $x10^{-3}$ mm^2/s
Mean tract-averaged value (95% CI)	0.22 (0.14 to 0.30)	1.473 (1.287 to 1.659)	0.27 (0.21 to 0.33)	1.167 (1.038 to 1.296)	0.29 (0.23 to 0.35)	1.216 (1.073 to 1.359)
Mean ROI value (95% CI)	0.38 (0.22 to 0.54)	1.371 (1.050 to 1.692)	0.42 (0.30 to 0.54)	1.032 (0.930 to 1.130)	0.43 (0.33 to 0.53)	1.031 (0.949 to 1.113)
Mean difference	-0.17	0.101	-0.15	0.136	-0.13	0.184
95% limits of agreement	-0.01 to -0.32	-0.21 to 0.415	-0.24 to -0.05	0.005 to 0.267	-0.22 to -0.05	0.062 to 0.307
Pearson correlation *r*	0.24	0.34	0.54	0.38	0.47	0.53
p value for correlation	0.032	0.002	<0.001	<0.001	<0.001	<0.001

Table 3. Intra-rater agreement for dMRI parameters generated by ROI placement in genu of corpus callosum and corticospinal tracts

	gCC		Right CST		Left CST	
	FA	MD $x10^{-3}$ mm^2/s	FA	MD $x10^{-3}$ mm^2/s	FA	MD $x10^{-3}$ mm^2/s
ROI Measure 1 / Mean (95% CI)	0.38 (0.22 to 0.54)	1.38 (1.01 to 1.75)	0.423 (0.31 to 0.54)	1.033 (0.94 to 1.13)	0.43 (0.33 to 0.53)	1.031 (0.95 to 1.11)
ROI Measure 2 / Mean (95% CI)	0.39 (0.21 to 0.57)	1.39 (1.08 to 1.70)	0.421 (0.30 to 0.54)	1.031 (0.93 to 1.13)	0.42 (0.32 to 0.52)	1.024 (0.93 to 1.12)
Mean difference	-0.001	-0.005	-0.0005	0.003	0.004	0.002
95% limits of agreement	-0.08 to 0.08	-0.3 to 0.29	0.08 to 0.08	-0.08 to 0.08	-0.06 to 0.07	-0.1 to 0.1
ICC / Mean (95% CI)	0.94 (0.9 to 0.96)	0.78 (0.65 to 0.86)	0.86 (0.78 to 0.91)	0.79 (0.67 to 0.86)	0.86 (0.78 to 0.91)	0.56 (0.32 to 0.72)
p value	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001

Figure 1. a: reference tract of genu of corpus callosum calculated from term controls displayed in green and overlaid on an age-specific standard space template b: illustration of segmentation of whole genu for an individual subject calculated using PNT c: ROI placement in genu of corpus callosum d: reference tract of right CST e: reference tract of left CST f. ROI placement in right and left posterior limb of internal capsule

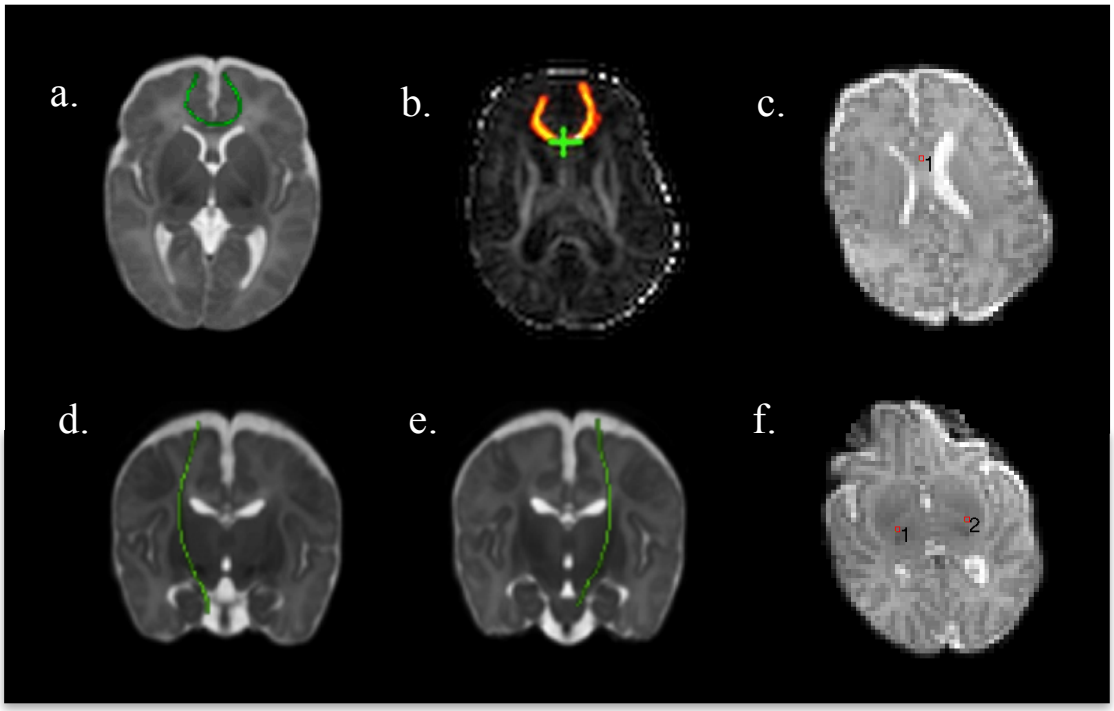

Figure 2. Axial (a), coronal (b) and sagittal (c) views showing genu, splenium and bilateral corticospinal tracts (scarlet) segmented using PNT within 3D space for a representative subject. Smaller tracts in 3D space running left / right are indicated in red, superior / inferior in blue and anterior / posterior in green.

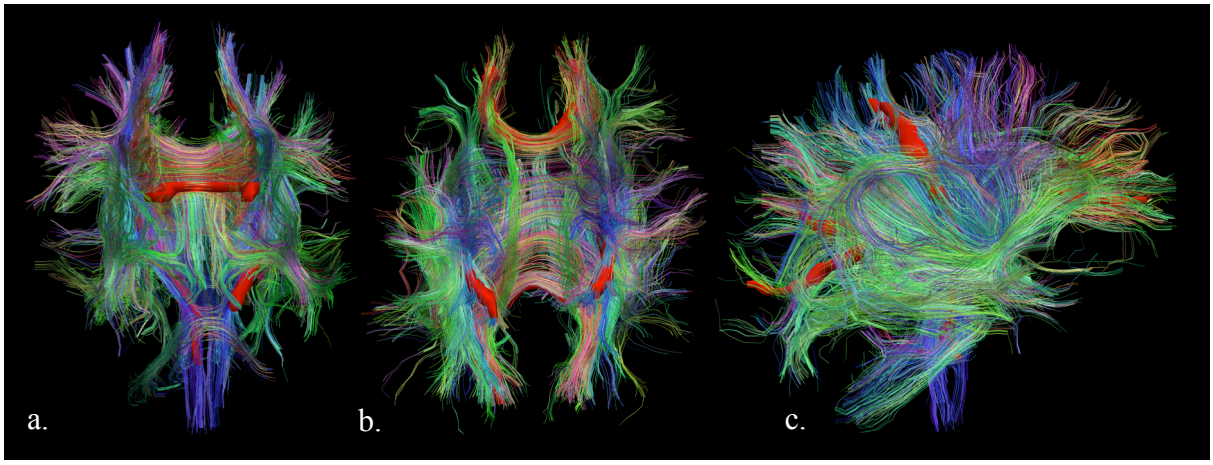

Figure 3. Bland-Altman plots of tract-averaged and ROI dMRI measurements in genu of corpus callosum and right and left corticospinal tracts.