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RESEARCH ARTICLE

Reproducibility of diffusion tensor imaging-derived parameters: implications for the streptozotocin-induced type 1 diabetic rats

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Abstract

Objective Diffusion tensor imaging (DTI) is a useful approach for studying neuronal integrity in animals. However, the test–retest reproducibility of DTI techniques in animals has not been discussed. Therefore, the first part of this work was to systematically elucidate the reliability of DTI-derived parameters in an animal study. Subsequently, we applied the DTI approach to an animal model of diabetes in a longitudinal manner.

Materials and methods In Study 1, nine rats underwent two DTI sessions using the same scanner and protocols, with a gap of 4 weeks. The reliability of the DTI-derived parameters was evaluated in terms of sessions and raters. In Study 2, nine rats received a single intraperitoneal injection of 70 mg/kg streptozotocin (STZ) to develop diabetes. Longitudinal DTI scans were used to assess brain alterations before and 4 weeks after STZ administration.

Results In the test–retest evaluation, the inter-scan coefficient of variation (CoV) ranged from 3.04 to 3.73% and 2.12–2.59% for fractional anisotropy (FA) and mean diffusivity (MD), respectively, in different brain regions, suggesting excellent reproducibility. Moreover, rater-dependence had minimal effects on FA and MD quantification, with all inter-rater CoV values less than 4%. Following the onset of diabetes, FA in striatum and cortex were noted to be significantly lower relative to the period where they had not developed diabetes (both $P < 0.05$). However, when compared to the control group, a significant change in FA caused by diabetes was detected only in the striatum ($P < 0.05$), but not in the cortex.

Conclusion These results demonstrate good inter-rater and inter-scan reliability of DTI in animal studies, and the longitudinal setting has a beneficial effect on detecting small changes in the brain due to diseases.

Keywords Reproducibility · Fractional anisotropy · Hyperglycemia · Longitudinal

Introduction

Diffusion tensor imaging (DTI) is a magnetic resonance imaging (MRI) technique that provides vital information on the structural properties of white matter (WM), based on the Gaussian distribution of water diffusion processes. With a diffusion tensor reconstructed from various diffusion gradient directions, DTI-derived parameters, such as fractional anisotropy (FA) and mean diffusivity (MD), have been identified as useful biomarkers for demyelination and

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axonal degradation in affected brain regions [1]. In addition, the reliability and reproducibility of the DTI technique have been firmly established in clinical practice [2, 3], making the DTI technique a part of clinical practice and offering the promise for longitudinal observational studies [4, 5].

Preclinical animals are essential for a better understanding of neuroscience research, offering the potential for findings to pose significant clinical implications. Therefore, there is growing interest in using animal DTI experiments to study neuronal integrity. Existing studies also emphasize the importance of longitudinal follow-up for individual animals [6, 7]. Despite its potential, the unexplored aspect of earlier animal studies was that the test–retest reproducibility of DTI-derived parameters in animals has not been discussed previously in the literature. Inter-scan reproducibility is affected by a combination of technical instability and physiological noise. Before its wider application in large-scale studies and proper interpretation of results, the reliability of FA and MD from DTI techniques in the animal studies needs to be assessed and validated. Therefore, the first part of this work was to systematically elucidate the reliability of DTI-derived parameters in terms of sessions and raters in an animal study.

Once the reproducibility of the DTI technique in the animal model has been validated, the second part of this study will apply this DTI technique to an animal model of diabetes in a longitudinal manner. It is well documented using DTI that the diabetic brain is associated with the significant brain alterations. However, the existing animal studies are cross-sectional experimental designs [8, 9], which are prone to inter-subject variations. Extending previous animal diabetes studies based only on measurements during a single, discrete post-diabetes period, the longitudinal experimental design in this study may further reveal information on diabetes-related alterations in brain function without interference from inter-subject variations.

Materials and methods

General

Eighteen female Sprague–Dawley (SD) rats (8 weeks old) were used in this study. The animals were housed under a temperature- and humidity-controlled conditions with a 12-h light–dark cycle and provided with a standard rodent diet and water ad libitum. All animal protocols were approved by the Institutional Animal Care and Use Committee. All MRI experiments were conducted using a 7 T animal MRI scanner (Bruker ClinScan 70/30, Germany) with a gradient strength of 630 mT/m. During MRI scans, the animals were anesthetized using medical air (1.0 L/min) with isoflurane (1.0–1.5%). Their body temperatures were maintained using a warm water circulation system and were measured using a rectal probe. Physiological parameters, including heart and breath rate, were recorded during the experiments (SA instruments Inc., NY, USA).

Study 1

The goal of this study was to investigate the test–retest reproducibility of DTI-derived parameters on the same scanner but on different days. Nine animals were used in this study and were designated as the control group. Each rat underwent two DTI sessions using the same scanner and protocols, with a gap of 4 weeks. For the MRI experiment, scout images along the three orientations were acquired using a T1-weighted sequence. Diffusion weighted images (DWIs) were obtained using a twice-refocused spin-echo echo planar imaging from the default setting of multi-directional diffusion weighting mode. The scanning parameters were as following: repetition time (TR)/echo time (TE) = 6000 ms/32 ms, flip angle = 90°, field of view (FOV) = 35 × 35 mm^2, matrix size = 128 × 128, nine axial slices, thickness = 1.5 mm, 30 gradient directions, 4 b values of 0, 500, 1000, and 1500 s/mm^2, and three averages.

Study 2

In this study, a longitudinal DTI experiment was used to evaluate brain microstructures before and after the onset of diabetes. An additional nine animals were used in this study and were referred to as the diabetic group. Animals in the diabetic group were intraperitoneally injected with a single dose of 70 mg/kg of streptozotocin (STZ, Sigma Chemical Co., St. Louis, MO) [10] at 8 weeks of age. After injection, non-fasting plasma glucose levels and body weights were monitored weekly for the next 4 weeks. This time span was consistent with previous studies showing that the brain exhibits significant diabetes-related alterations 4 weeks after STZ administration [8, 9]. Rats with non-fasting plasma glucose levels > 250 mg/dL were considered as type 1 diabetic rats and used for the following study. As the measurement range for the glucometer (Accu-Chek, Basel, Switzerland) is 10–600 mg/dL, animals with particularly high plasma glucose levels reaching the top limit can only be recorded as 600 mg/dL. One diabetic animal died during the follow-up period, leaving eight animals in the diabetic groups. The MRI scans were longitudinally performed on all animals before and 4 weeks after STZ administration. The imaging parameters were identical to those used in Study 1.

Data analysis

DTI data from one rat in the control group and two rats in the diabetic group were excluded due to motion artifacts

or poor image quality. As a result, the DTI data from eight and six rats in the control and diabetic groups, respectively, were used for the final analysis. The raw DTI images were first realigned to the non-diffusion-weighted b0 image. Then, DTI-derived parameters, such as FA and MD, were estimated using the DSI Studio [11]. The diagonalization of the diffusion tensor generated three eigenvalues, λ_1, λ_2, andλ_3, where FA and MD were computed. Regions-of-interest (ROIs) of the corpus callosum, cortex, and striatum were delineated manually from the EPI images of each animal at each time point, as shown in Fig. 1. Based on previous DTI animal studies on diabetes, these regions were the key regions involved in diabetes-related abnormalities [8, 9]. The resulting ROI masks were then applied to the FA and MD maps to estimate regional values by averaging the values from all voxels in the ROI mask. Additionally, as the data processing involved manual ROI selection, the inter-rater reliability of DTI data analysis was evaluated by having two raters that independently analyze the same datasets as in Study 1. Rater 1 had 7 years of experience in ROI drawing in terms of animal studies, and another rater was trained by Rater 1 for the purpose of this study.

Histological assessments

After the MRI scans were completed, the rats were sacrificed and perfused with 250 ml fixative (4% paraformaldehyde in 0.1 M phosphate buffered saline, pH 7.4). Cerebral tissues were kept in 10% formalin and embedded in paraffin wax for histological assessment. Serial 5 µm cross-sections were cut and stained with hematoxylin and eosin (H&E).

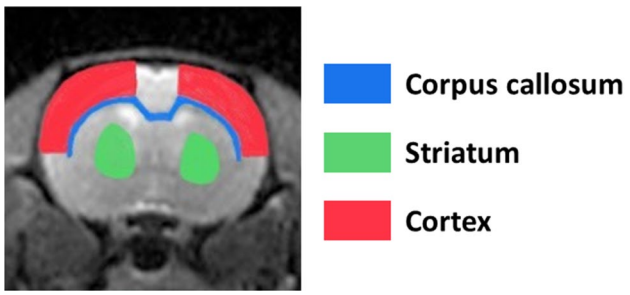

Fig. 1 Brain parcellation illustrating the brain regions in the region-of-interest analysis on the echo planar imaging

Statistical analyses

All statistical analyses were performed using MATLAB and visualized using Excel. The Bland–Altman plot [12] was used to visualize the agreement of the DTI-derived parameters obtained from different days and raters in Study 1. Variability was evaluated using the coefficient of variance (CoV), given by the standard deviation across measures divided by mean values. A paired *t* test was used to determine whether alterations in FA and MD were significantly different after diabetes onset in Study 2. To assess region-specific differences between the control and diabetic groups, the Student's *t* test was applied to pairs of studied groups of regional FA and MD values. Statistical significance was set at $P < 0.05$.

Results

Study 1

Representative diffusion weighted images (DWIs) using different b-values at two time points are shown in Fig. 2. Notably, because the signal intensity in DWIs can depend on the diffusion gradient direction, the DWIs with diffusion gradients were the average of 30 gradients with different directions. As can be seen, the signal intensities decreased substantially with an increase in the b-values. Nevertheless, the image quality was sufficient to distinguish anatomical structures, such as the corpus callosum, suggesting the reliable image quality used in this study. Visual inspection also suggested comparable image quality between the two time points. The corresponding signal-to-noise ratio (SNR) for each of the brain regions investigated in this study at each b-value is shown in Supplementary figure.

Figure 3 shows the Bland–Altman plots of FA and MD between the two raters. The inter-rater CoVs ranged from 1.21 to 3.49% and from 0.37 to 1.45% for FA and MD in

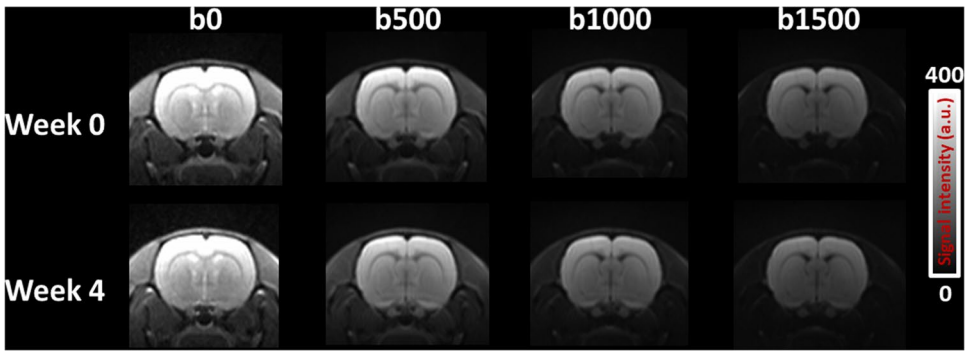

Fig. 2 Representative diffusion weighted images using different b-value settings at two time points

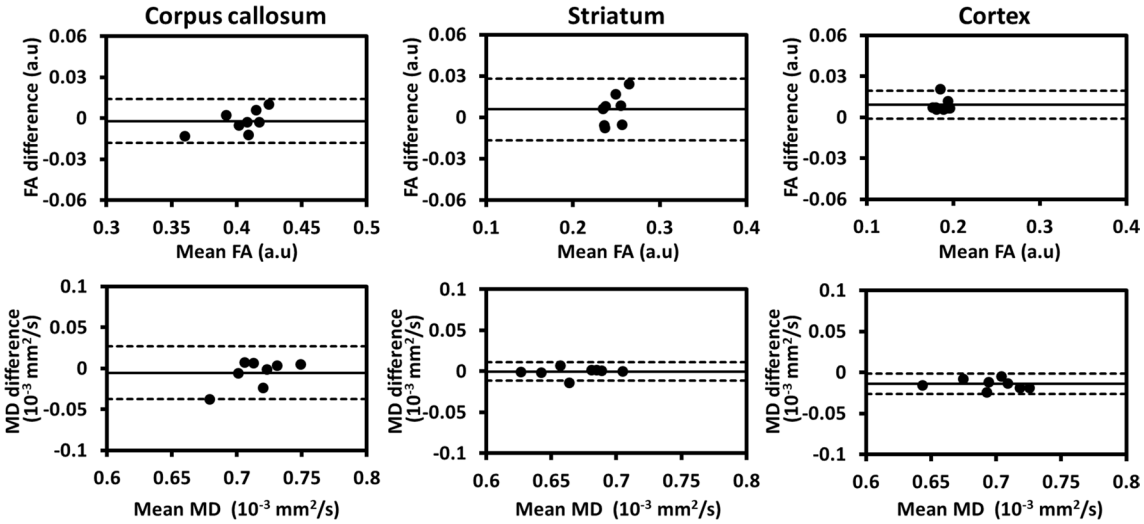

Fig. 3 Bland–Altman plots of DTI-derived parameters obtained from two raters in the inter-rater reliability study. The solid line indicates the mean difference between two raters, and the dashed lines indicate the 95% confidence interval. Each dot represents data from each animal. *FA* fractional anisotropy, *MD* mean diffusivity

different brain regions, respectively. Our results demonstrated that there was close agreement in the FA and MD measurements between raters. Figure 4 shows the Bland–Altman plots of FA and MD between the two sessions. The inter-scan CoVs ranged from 3.04 to 3.73% and 2.12–2.59% for FA and MD, respectively, in different brain regions. All CoV values were below 5% for the DTI-derived parameters, suggesting the excellent reproducibility of the measurements. As the FA/MD variations caused by different raters were smaller than the between-day variations, the rater effect was not a major contributor to the uncertainty in the FA/MD quantification. The inter-rater and inter-scan CoVs of these measures are shown in Table 1.

Study 2

The weight and plasma glucose concentrations of the diabetic animals are shown in Fig. 5a, b, respectively. After receiving STZ injection, the animals exhibited the diabetic characteristic of increasing plasma glucose concentration. Notably, all animals developed glucose values higher than the measurable range (> 600 mg/dL) 2 weeks after STZ

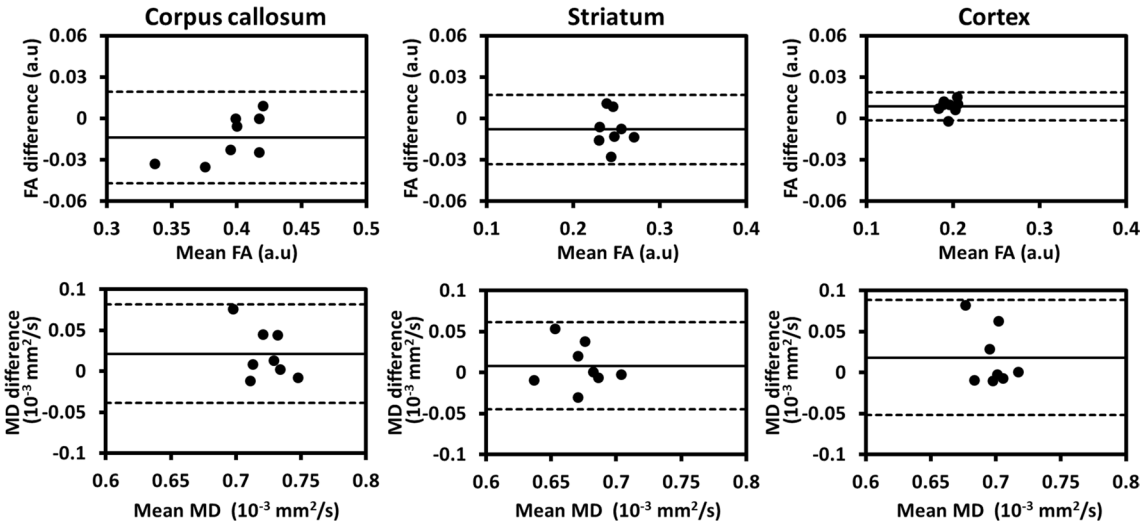

Fig. 4 Bland–Altman plots of DTI-derived parameters obtained from different day in the inter-scan reproducibility study. The solid line indicates the mean difference between two raters, and the dashed lines indicate the 95% confidence interval. Each dot represents data from each animal. *FA* fractional anisotropy, *MD* mean diffusivity

Table 1 Summary of the inter-rater and inter-scan reproducibility of the study

	FA		MD	
	Inter-rater	Inter-scan	Inter-rater	Inter-scan
Corpus callosum	1.21 ± 0.82	3.03 ± 2.79	1.15 ± 2.39	2.58 ± 2.62
Striatum	2.92 ± 1.77	3.73 ± 2.02	0.37 ± 0.48	2.12 ± 2.03
Cortex	3.49 ± 1.97	3.33 ± 1.43	1.45 ± 0.64	2.59 ± 3.17

The data are expressed in mean and standard deviation for the associated CoV (%)

injection, and plasma glucose can only be recorded as 600 mg/dL.

Figure 6(a, b) shows the results of the ROI analysis of FA and MD at the group level, respectively. Following the onset of diabetes, FA in striatum and cortex were noted to be significantly lower when compared with when they had not developed diabetes (both $P < 0.05$). However, compared to the control group, a significant change in FA caused by diabetes was only detected in the striatum ($P < 0.05$). There was no apparent difference in cortical FA between the control and diabetic groups ($P = 0.27$). The MD was found to be unchanged before and after the onset of diabetes across different brain regions. Moreover, MD analysis failed to detect any significant differences between the groups (all $P > 0.05$). FA and MD were not significantly different between two time points in the control group, suggesting that FA and MD remained constant this age range.

Figure 7 shows light microscopic examinations of H&E–stained sections of the brain tissue of control and diabetic groups. Compared to the control, most cells were irregular in shape with shrunken deeply stained nuclei in the diabetic animals. The results of H&E staining demonstrated the brain injury in the diabetic group.

Discussion

In this study, we conducted a series of experiments to assess the reliability and reproducibility of DTI-derived parameters. Our results showed that the DTI-derived parameters of FA and MD achieved a high level of competence in inter-scan reproducibility over time. This excellent reproducibility could benefit the longitudinal DTI experiments using the same rat. Although the DTI results involved manual selection of the ROI, the rater-dependence had minimal effects on FA and MD quantification. Unlike previous DTI studies on animals with diabetes [8, 9], this is the first study to perform longitudinal DTI experiments before and after the onset of diabetes. It has been shown that animals exhibit decreased FA in the striatum and cortex after the onset of diabetes, providing useful information regarding disease progression without interference from inter-subject variations.

Assessment of the test–retest reproducibility of DTI-derived parameters is significant for longitudinal studies, as it is crucial to interpret longitudinal changes caused by intra-subject variations. Many elements contribute to measurement variability, and physiological variations and MRI

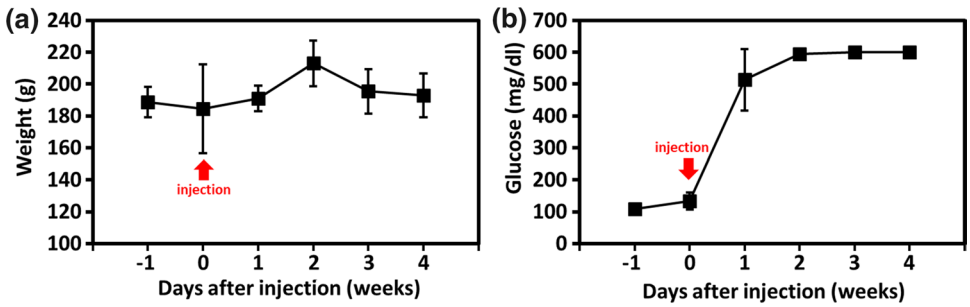

Fig. 5 Weekly (**a**) weight and (**b**) plasma glucose level measurements for animals in the diabetic group

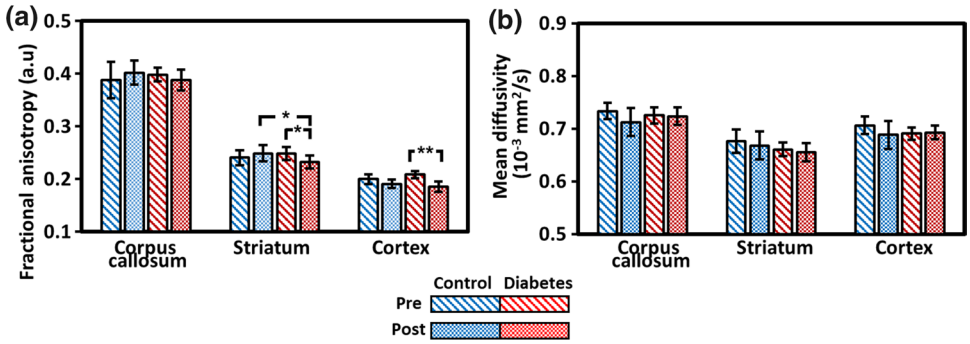

Fig. 6 Diffusion tension imaging derived-parameters of (**a**) fractional anisotropy and (**b**) mean diffusivity in the regions of interest averaged across rats between groups. *: $P < 0.05$; **: $P < 0.01$

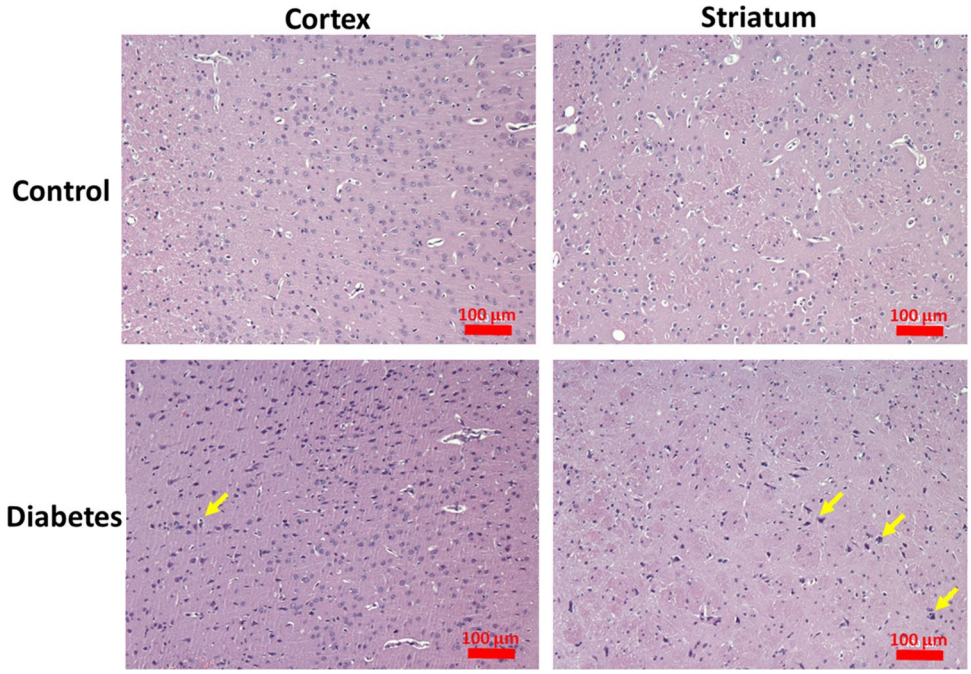

Fig. 7 Representative hematoxylin and eosin staining for cortex and striatum. Yellow arrows indicate damaged neurons

system instability are key elements to consider. Although DTI studies conducted at higher field strengths, such as 7 T, produce better image quality, the susceptibility-related effects increase linearly with the magnetic field strength [13]. Previous studies have suggested that susceptibility-related background gradients can interfere with diffusion-encoding gradients, jeopardizing the quantification of molecular mobility [14]. Moreover, the different levels of magnetic field homogeneity and the orientation of the white matter fibers when repositioning the animals may affect the direct comparisons between days. However, our results showed that the inter-scan CoV ranged from 3.04 to 3.73% for FA with a 4-week interval. These CoV values were considered good when compared with clinical studies, with CoV values ranging from 0.8 to 5.9% [2, 3], suggesting robust and reliable data acquisition in animal DTI experiments. The comparable test–retest reproducibility in our 7 T study may be attributed to the pulse sequence of the twice-refocused spin echo (the default setting in the multi-directional diffusion weighting sequence) used in this study, which has been shown to compensate for the effects of field inhomogeneities in the diffusion data [15]. Reproducible DTI measurements may also imply that the DTI technique is feasible in populations of rats aged 8–12 weeks. Further age-related corrections were not required in rodent DTI studies. These temporal behaviors in the DTI-derived parameters are in agreement with the findings of Bockhorst et al. showed that both FA and MD did not change over time after postnatal day 28 [16]. Of further note, to comply with the experiments, the use of anesthetics is inevitable in animal studies. It is known that both the agents used and the concentration employed have significant impacts on anesthetic depth [17, 18]. Therefore, it is possible that the reliability of DTI measurements was also dependent on the anesthesia regimes and dosages, which were not accounted for in the present study.

Variations between sessions are a topic of interest when planning longitudinal studies for the same subjects, while another important aspect of the reliability of the DTI quantifications is the inter-rater variations, as DTI results involve the manual selection of ROI. With written instructions and short training, an inexperienced rater was able to process the DTI data independently. Although the delineation of ROIs without the assistance of a template may be a source of errors and lead to data variability, our results showed that the inter-rater CoVs were all less than 4% and smaller than the inter-scan CoVs. The excellent inter-rater precision suggests that rater-dependence has minimal effects on DTI quantification, and guarantees the robustness of this method. Notably, the second rater was trained only by rater 1. Although experience gained from the existing clinical studies also suggests that comparing the performance of experienced and inexperienced raters (the inexperienced rater was also trained by the experienced rater) is important for evaluating the training procedure [19, 20], the involvement of other relevant experienced raters would have been desirable to evaluate the measurement variability among raters.

To extend the applicability of this DTI approach for studying brain perturbations related to diseases, we evaluated the validity of this method in the diabetic brain in an animal model. Our results demonstrated that diabetic animals exhibited reduced FA in the striatum and cortex compared with those without diabetes development in the longitudinal

setting. However, there was no apparent difference in the cortical FA between the control and diabetic groups. STZ-induced animals that developed reduced FA in the striatum and cortex have also been demonstrated by Huang et al. in a cross-sectional design, which used 16 and 24 animals in the control and diabetic groups, respectively [8]. These findings suggest that the longitudinal design and within-subject comparisons in this study have the beneficial effect on eliminating inter-animal variation, thereby increasing the detection power, allowing us to use a sample size half of that of the prior study to detect smaller changes due to diseases. This evidence may also tentatively suggest that the same longitudinal DTI technique should be useful for studying the progress of brain complications associated with diseases or for detecting therapeutic effects over time within the same animal.

MD is another valuable index derived from DTI techniques for evaluating disease-related microstructural changes. In parallel to this concept, a series of studies have used MD to track the impact of diabetes on brain tissues, and these results concluded that patients with diabetes have significantly elevated MD in several brain regions [21, 22]. Surprisingly, we found no evidence of significant changes in MD in diabetic animals, similar to the large-scale clinical study conducted by Liu et al. [23] The *P* values were 0.86 and 0.31 for cortex and striatum, respectively. These *P* values were far from the significance level, and expanding the sample size may not beneficially increase the statistical power. As MD is susceptible to cellularity and edema [24], the unchanged MD observed in this study may suggest that the cerebral edema did not develop as early as 4 weeks after the onset of diabetes [8].

FA has been widely considered a surrogate for WM microstructural integrity, but we did not find any significant FA changes in the major WM tracts, such as corpus callosum, in the diabetic animals. The insignificant change in FA is in general agreement with an animal study reported by Huang et al. [8], but inconsistent with the results reported by Ding et al. [9]. One possible reason for this may be related to the use of different animal models of diabetes. The diabetic animal model used in this study and in Huang et al. was type 1 diabetes, whereas the one used by Ding et al. was type 2 diabetes. Although both type 1 and type 2 diabetes share the common characteristic of persistent hyperglycemia, they have distinct etiologies and pathophysiological effects. Therefore, different types of diabetes may exhibit different patterns of brain alterations [25]. Another possible explanation for this discrepancy is with respect to the pulse sequences and image-processing techniques. DTI measurements are susceptible to variations in acquisition parameters [26], data-processing strategies, and analysis software [27, 28]. To better understand the underpinnings responsible for the heterogeneity observed across studies, the same dataset analyzed using different scanning parameters and strategies would hopefully untangle these controversies.

The results of this study have several limitations. First, in this study, we only discussed the inter-scan reproducibility of DTI techniques with an interval of 4 weeks. For the wide application of this DTI approach to other brain diseases, assessment of long-term reproducibility should be further explored and investigated. Second, only H&E staining was used to examine the histological changes. H&E staining is one of the gold standard tissue stains used in histology to assess brain damage [29], and alterations in the diabetic brain proved by H&E staining have been demonstrated in our and other studies [19, 30]. Nevertheless, further sensitive and delicate immunohistochemistry techniques such as Luxol fast blue staining [31] are needed to uncover demyelination or axonal/neuronal damage in the diabetic brain to comply with the findings of DTI studies. Third, only female animals were included in this study. Sexual dimorphism in diabetes-related brain alterations has been reported in the literature, with diabetic females exhibiting prominent brain atrophy [32] and compromised brain metabolism [10] compared to their male counterparts. In the case of sex-related differences in brain alterations using DTI techniques, experimental designs similar to the current one but extended to different sexes or even different strains of animals are of paramount importance in future studies. The last limitation is related to spatial resolution. Although the voxel size used in this study was similar to that used in a previous DTI diabetes study [8], a partial volume effect was inevitable. It has been shown that voxels with cerebrospinal fluid contamination lead to an underestimation of FA [33]. The partial volume effect could have caused the group comparisons to be incorrect. Decreasing the voxel size is the most obvious approach for minimizing the partial volume effect. However, this compromises the image SNR, leading to another accuracy problem.

In conclusion, DTI technique is capable of repeated measurements in animal studies. It can provide reproducible DTI metrics in the rat brain at an interval of four weeks, and the results were shown to be rater-independent. Moreover, extending the applicability of this DTI approach for studying brain perturbations related to diseases, longitudinal experiments have a beneficial effect on distinguishing FA changes due to diabetes. These findings suggest that DTI-derived parameters can be reliable imaging biomarkers for studying neuronal integrity and provide an important reference for its applications in future longitudinal animal studies.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s10334-022-01048-w.

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Data Availability The datasets generated and/or analysed during the current study are not publicly available due to ethical issues but are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no conflict of interests.

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