Deterministic tractography analysis of rat brain using SIGMA atlas in 9.4T MRI

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01 Introduction

- Preclinical studies using rodent studies, which are known to reflect human biology, have been the choice for many neuroscience researchers.
- In particular, research using **magnetic resonance imaging (MRI)** can accurately identify brain regions by acquiring high cavity resolution and different contrasts, acquire different tissue and characteristic information, and is non-invasive, high-throughput and high reproducibility.
- In addition, tractographic analysis using **diffusion tensor imaging(DTI)**, which can obtain information on the neural structure of white matter, has emerged as a major methodology in the field of neuroscience because partially altered neural connections can contribute to various neurological and psychiatric diseases.
- However, unlike image analysis studies with human subjects, where various programs have been developed and validated to provide a complete assessment and step-by-step work procedure, methods for analyzing image data using MRI in preclinical research settings have not been agreed upon.
- Therefore, in this study, for the high-accuracy **SIGMA atlas**, we present a deterministic tractographic analysis pipeline that can perform detailed structural segmentation of the rat brain and confirm structural connectivity based on the segmented regions.
- In addition, using the analysis pipeline presented in this study, **structural connectivity analysis** was performed by applying it to the stroke Rat model image data analysis.

02 Materials and Methods

Preparation of Animals

- Analysis methods were applied on **1 normal rat and 7 middle cerebral artery occlusion (MCAO) model rats. 3-month-old male Sprague-Dawley rats (SD, Orient Bio, Seoul, Korea) weighing 250-350 g** were used in this experiment.

Animal models

- The animal model of central cerebral artery occlusion (MCAO) induced by focal cerebral infarction was performed using a previously known method.
- SD rats were anesthetized by the exposure of 1.5 to 2% isoflurane, the right common carotid artery was exposed, and the external and internal carotid arteries were separated.
- After incision of the external carotid artery, which is 7 to 8 mm from the bifurcation, the external carotid artery was placed in a straight line with the internal carotid artery.
- A 4-0 black monofilament suture coated with silicone (diameter: 350㎛) was inserted to the puncture site of the external carotid artery (403656PK10, Doccol Cooperation, USA) and move toward to the origin of the middle cerebral artery by passing through the internal carotid artery.
- After 90 minutes of MCAO, inserted filament was retrieved for the reperfusion of cerebral blood flow.
- During the surgery and recovery period, the temperature was adjusted to 37.0±0.5℃ with a thermostat using a heating pad.

MRI acquisition

- Image data acquired in this study were performed on a **9.4T Bruker BioSpec horizontal bore animal scanner equipped with a tilt system of (660 mT/m)**.
- The image data collection of normal rats was performed at the **Cell to In-invo imaging Core-facility Research Center (CII, Gachon University, Lee Gil-ya Cancer Diabetes Research Institute)**, and the image data collection of the MCAO model was performed at **Sungkyunkwan University N Center (IBS, institute for Basic Science, Suwon, Korea)**.
- The pulse sequence used for this acquisition was a **2D EPI-diffusion tensor**,

Figure 1. BioSpec Advance 94/20 USR.

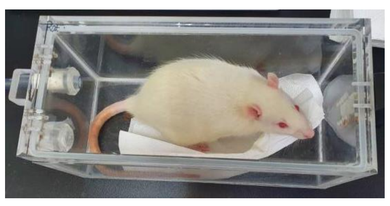

Figure 2. SD RAT.

Normal Rat

Spin echo sequence with a repetition time = 2500 ms, echo time = 21.3165 ms, flip angle = 90°, bandwidth = 170 kHz, b-value = 2011.85 s/mm², diffusion gradient pulse duration (δ) = 4.5 ms, diffusion gradient separation (Δ) = 10.6 ms, diffusion direction = 30, field of view = 2.5 × 3.5 cm, slice thickness = 0.4 mm, matrix = 125 × 175 , slice = 40, resolution = 200 x 200 x 400 µm, 4 averages and resulting in a total acquisition time of 1 h 15 m 50s

Modeling Rat

Spin echo sequence with a repetition time = 3000 ms, echo time = 17.0505 ms, flip angle = 90°, bandwidth = 341 kHz, b-value = 1389.93 s/mm², diffusion gradient pulse duration (δ) = 2.5 ms, diffusion gradient separation (Δ) = 8.5 ms, diffusion direction = 30, field of view = 2.5 × 2.5 cm, slice thickness = 0.3 mm, matrix = 83 × 83, slice = 115, resolution = 301 x 301 x 300 µm, 2 averages and resulting in a total acquisition time of 28 m

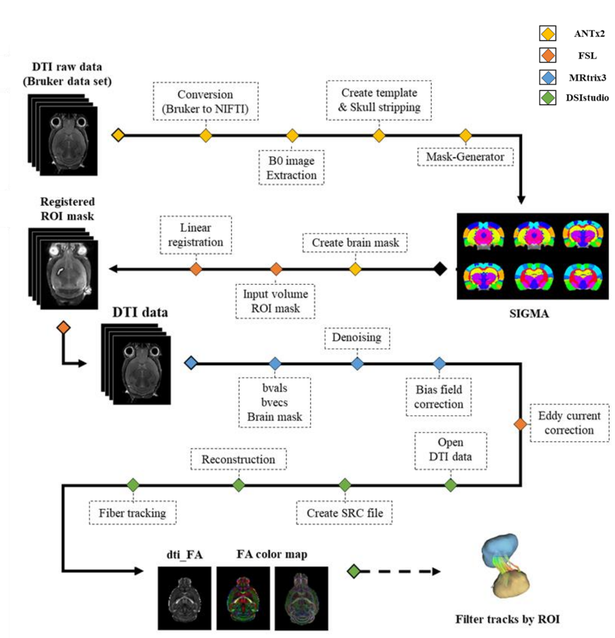

Figure 3. All analysis pipeline of the image data.

Image data processing

- The acquired DTI data was first processed via **ANTx2**. Data in Bruker format was converted to Neuroimaging Informatics Technology Initiative (NIFTI) format and normalized to **SIGMA** space. B0 images were extracted from the normalized image data with the skull and other tissues other than the brain removed. Brain and brain structure masks were acquired by segmenting each ROI used for analysis on the extracted b0 image data.
- The acquired ROI masks were registered to the DTI data using the **FMRIB software library version 6.0.2.** After all the masks were linearly registered to DTI space using the FLIRT function, the registered masks were qualitatively evaluated whether each mask was registered to the correct position.
- **MRtrix3** was used for the preprocessing of the DTI data. DTI data was denoised and bias field corrected to remove noise and correct for B1 field non-uniformity.
- Additionally, FSL's eddy correct was used to correct for distortions and motion artifacts. The preprocessed data were used for deterministic tractography analysis in **DSIsudio**. After the DTI data was converted into SRC format, a range of brain voxels specified by the segmented masks was selected for fiber orientation reconstruction and fiber tracking.

Deterministic Tractography

- Tractography of ROIs were obtained using DSIstudio's **Q-sampling imaging (GQI)**, which involves decomposing up to two fibers in one voxel by a wireline tracking algorithm.
- A deterministic streamlined tracking algorithm with high connectivity validation were reconstructed through each ROI selected from the **SIGMA atlas (M1, M2, S1, S2, CC, IC and CP)**.
- Each reconstructed fiber that passes through the ROI, enters that region and does not proceed further. The fiber tracking (Tracking Threshold: 0.1, Angular Threshold: 45º, Step Size: 1.5, Min Length: 0.5, Max Length: 250, Terminate if: 2000000) process results in the number and shape of the streamlines passing through the target area.

03 Results

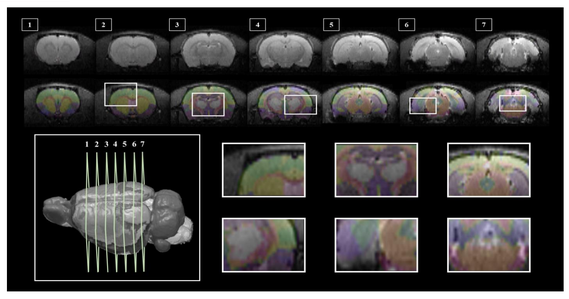

Figure 4. SIGMA Atlas-based Whole Brain Segmentation and Enrollment Results. Slices (1–7) shows the location of the segmentation result overlaid on the image data, 3D rendered RAT brain (bottom left) to show each slice location, and 6 detailed views shows atlas overlays (bottom right).

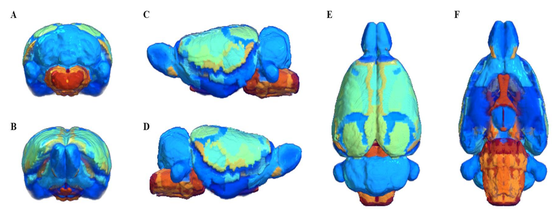

Figure 5. 3D rendering of segmentation and registration results. posterior (A), anterior (B), left lateral (C), right lateral (D), superior (E), and inferior (F). Regions are colored to identify their boundaries, and color similarity between spatially separated regions is meaningless.

SIGMA Atlas-Based Whole Brain Segmentation and Registration

- In order to qualitatively verify the accurate segmentation information of detailed structures, the segmented brain structure region on the B0 image data is visualized in **Figure 4.**
- The division and registration of all structures appears to be clearly registered at each location based on the SIGMA atlas, and it can be confirmed that even strong deformations of anatomical structures are outlined realistically by the algorithm.
- The segmentation and registration results are 3D rendered and presented in **Figure 5** so that location information and shapes can be checked from various directions.

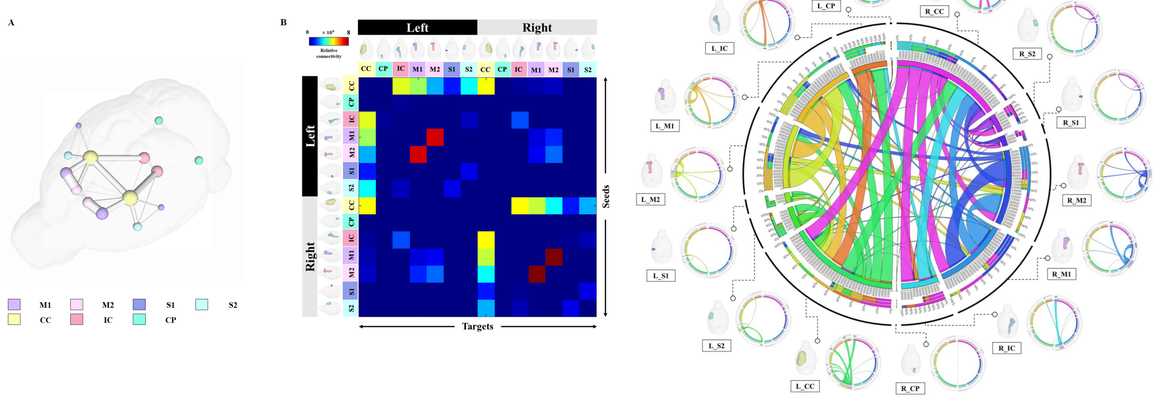

Figure 6. Results of tractography analysis between 14 anatomical regions. The connectivity between each structural region in the 3D rendered whole brain is shown in (A), and the label of each structural region is shown at the bottom of the figure. In addition, the connectivity matrix between each structural region was plotted using (B) connectivity strength color maps with the seed plotted on the left and the target plotted on top

Figure 7. Connection diagram showing the structural connectivity of the consensus among the 14 structural domains. The structural regions of each cluster are represented by rectangles around a large circle, and the lines connecting the rectangles indicate the connections between the corresponding structural regions. The thicker the line, the higher the connectivity, and the thinner the line, the lower the connectivity. The center circle is expressed as the sum of the strengths of the connections between all structural regions, and the diagrams from each structural region (seed) to the other structural regions (target) are expressed around the circle.

Deterministic tractographic analysis

- For structural connectivity, seven regions (M1, M2, S1, S2, CC, IC, CP) related to the corticospinal tract (CST) that transmit movement-related information from the cerebral cortex to the spinal cord were segmented and registered.
- All segmented and registered structural regions were subdivided into the left and right hemispheres, and connectivity between the 14 regions were generated. Each structural region was divided into seed and target, and deterministic tractographic analysis was performed, with the results indicating the connectivity between each structural region are presented in **Figure 6** and **Figure 7**.

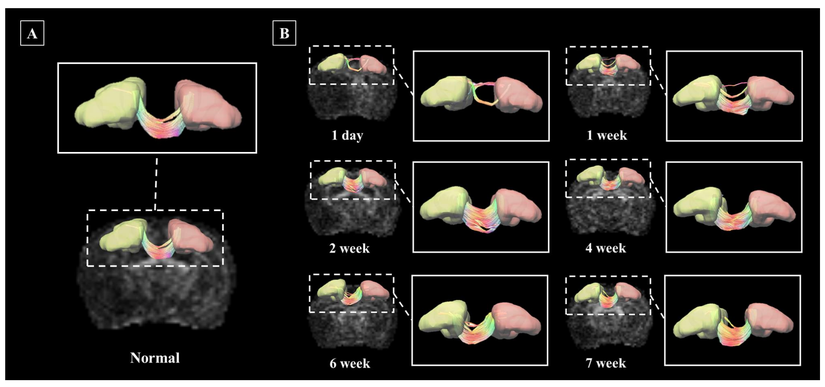

Figure 8. Nerve pathways in the Left M1 and Right M1 regions, shown in D rendering. Linkage pathways of normal rats (A), and linkage pathways by date of disease occurrence in a stroke rat model (B). Rendered structures and connection paths are represented as axial planes, and connection paths between structural regions are enlarged and presented in greater detail.

Application of deterministic tractographic analysis of stroke model

- The segmentation and deterministic tractographic analysis pipeline established in this study was applied to the stroke model.
- In the stroke Rat model, images were acquired once every 1 day, 1 week, 2 weeks, 4 weeks, 6 weeks, and 7 weeks after the onset of the disease, and disease progression was observed.
- **Figure 8** investigates structural connectivity in the motocortex of the left and right hemispheres known to suffer from stroke.
- **Figure 8-A** shows the neuronal pathways between Left M1 and Right M1 in unmodeled normal rats, and **Figure 8-B** shows the changes in the neuronal pathways between Left M1 and Right M1 from 1 day to 7 weeks after stroke onset.
- It was confirmed that the Left M1-Right M1 connection of the stroke model was significantly reduced on the 1st day compared to the normal, and it recovered gradually and showed a shape similar to that of the normal rat after the 2nd week.

04 Discussion

- We present a tractographic analysis pipeline that can determine the segmentation of detailed structural regions and connectivity based on structural regions using MRI image data of the rat brain.
- The pipeline efficiently combines a variety of existing neuroimaging analysis tools to enable structural segmentation and tractographic analysis.
- In addition, the entire brain template of Rat is provided using the highly accurate SIGMA atlas from which a researcher can acquire detailed structural region information through segmentation, as well as perform regional analysis of image data.
- By applying a pipeline to the acquired DTI data, we were able to successfully obtain the results of connectivity analysis between structural regions.
- We were able to quantitatively check the connectivity between each structural region, and generate 3D renderings of the connectivity strength, connectivity matrix, and connectivity pathways for comparison.
- In this study, we presented a structural analysis and structural connectivity analysis pipeline of the rat brain by efficiently combining various existing neuroimaging analysis tools. From the results, we were able to successfully extract and segment individual ROI masks, and perform tractgraphic analysis. The pipeline presented in this study can contribute to standardizing various data types and analysis methods in the field of neuroscience using preclinical animals, and can enable comprehensive application of structural analysis and structural connectivity.

Cozzi J et al., 2008; Shin JA et al., Journal of neuroimmunology, 2009; Jones DK et al., 2010; Badhwar A et al., 2013; Xue K et al., 2014; Lancelot S et al., 2014; Pallast N et al., 2019; Pallast N et al., 2019; Goerzen D et al., 2020 Lein ES et al., 2007; De Guzman AE et al., 2016; Huebner NS et al., 2017; Barrière DA et al., 2019; Koch S et al., 2019; Po C et al., 2012; Calabrese E et al., 2015; Andersson G et al., 2018; Sinke MR et al., 2018; Pallast N et al., 2020; Schilling KG et al., 2020; Trinkle S et al., 2021; Sarwar T et al., 2021