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IMAGING THINLY MYELINATED WHITE MATTER PATHWAYS

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Introduction

Crocodilians are a key species for understanding the evolutionary history of white matter pathways [1, 2]. They are the closest living relative to modern birds and they share an important ancestor with mammals [3]. Genomic mitochondrial evidence suggest that crocodilians and birds diverged approximately 240 million years ago [4], and they have not evolved significantly over that time [5]. This makes the crocodilians an advantageous species to study and learn how past white matter pathways functioned.

White matter pathways are made up of fibers tracts. These fiber tracts actively affect how the brain learns, how neurons communicate with one another, and how the brain regions work together in a coordinated fashion [6]. Visualizing these fiber tracts can be accomplished in multiple ways [7], [8]. However, this study used enhanced magnetic resonance imaging (MRI) and reconstruction techniques, namely diffusion tensor imaging (DTI) and tractography.

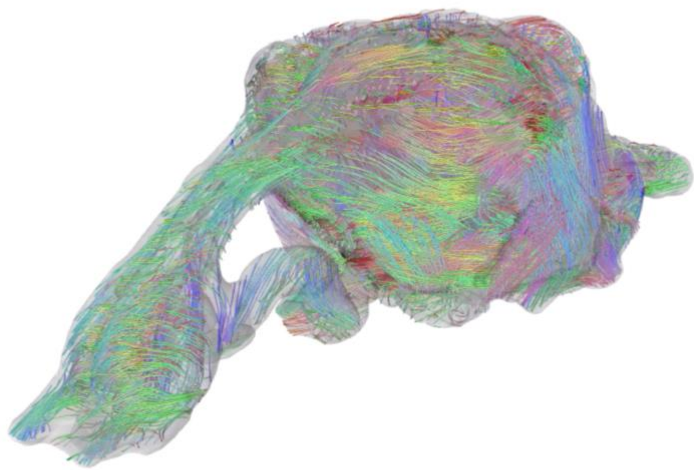

Figure 1: Three-dimensional reconstruction of fiber tracts in an American alligator brain using DTI. The colors represent which direction the tract is moving. Created using DSI Studio.

DTI selects the most prominent direction of water molecule diffusion to determine the orientation of axon fiber bundles. This method has emerged as the preferred means to visualize white matter structures in human brains [9]. It is a non-destructive alternative to conventional histology, meaning it can be done non-invasively and does not require animal euthanization [10]. Tractography is a computational reconstruction technique that uses discrete DTI datasets of axonal fiber orientations to create a continuous, three-dimensional fiber tract model [11]. It is assumed that DTI cannot accurately reconstruct neural pathways without thickly myelinated fibers [12] – [15]. To our knowledge, DTI has not been performed on crocodilians. There is experimental evidence showing that crocodilians have thinly myelinated fibers [16]. If DTI requires thick myelin to visualize fibers, then it would not be a viable method to visualize the white matter pathways in the crocodilian brain. In this study, we determined if thinly myelinated white matter pathways in an alligator brain could be visualized using DTI.

Methods

To determine if the thinly myelinated fiber tracts could be visualized using DTI and tractography, we studied the auditory tracts of one previously harvested and fixed juvenile American alligator brain. The auditory tract was chosen because previous experimental evidence has shown that the auditory tract in an alligator brain has thinly myelinated fibers [16] – [18]. A simplified diagram for this auditory pathway is illustrated in Fig. 2.

The DTI data was loaded into an open source tractography program and regions of interests (ROIs) were placed in different regions of the brain using experimental evidence [16] (Fig. 3).

After the ROIs were placed, one ROI was chosen to be a starting point, while the other was the target. For each experiment, the tractography program would stop tracing once it found several tracts connecting the two ROIs. To determine if the tractography reconstructions were correct, they were qualitatively compared to the experimental reference structure (Fig. 2) [16]. If the tracts went outside of the brain, they were considered to be artifacts and were deleted by using a cutting tool within the tractography program.

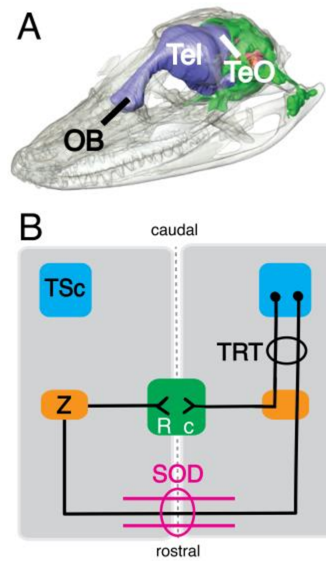

Fig. 2: Simplified diagram of ascending connections of the thalamic area in the alligator brain. The colored squares and circles represent different regions of the brain. The black lines represent which regions are connected together. Figure courtesy of MB Pritz, Ph.D.

Fig. 3: Three-dimensional representation of the locations of the ROIs in the alligator brain. The three-dimensional reconstruction was modeled using DSI Studio.

Results

The DTI results were visualized using an open source tractography program, DSI Studio (v. Fall 2018). As shown in Fig. 4, there are connections between all of the ROIs. The right image is a representation of the tracts in three-dimensional space showing the connectivity between the ROIs. The colors represent the direction of the tracts in XYZ space. Most of the tracts were well defined, meaning that almost all of the tracts moved in the same direction. The tracts originating from the top right blue ROI (TSc – refer to Fig. 2) did not have a direct path to the right orange ROI (Z). It spiraled around the TSc ROI before traveling to the Z ROI. This is pointed out by the blue arrow in Fig. 4.

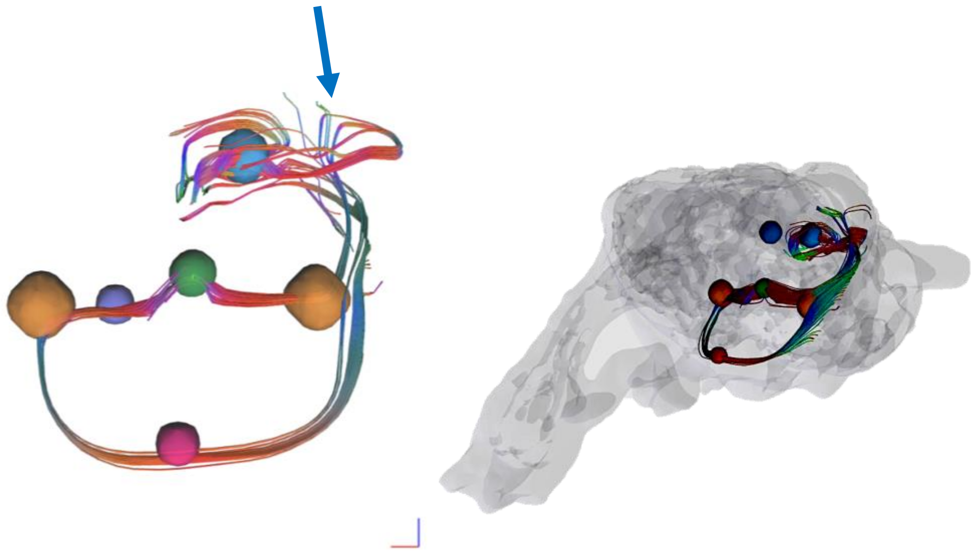

Fig. 4: DTI tractography results connecting the ROIs. Left: 2-D model. The blue arrow points to the area where the tract spiraled around the ROI. Right: 3-D model of same tract with the alligator isosurface brain surrounding it in grey.

Discussion

By combining DTI and tractography, we were able to model connections between different regions of the brain (Fig. 4). Our results revealed a spiral configuration of the tract around the right TSc (refer to Fig. 2 or Fig. 5) that did not align with the experimental reference structure. The spiral configuration (Fig. 4) may have been caused by misplacement of the ROI. The TSc ROI may have been placed in the wrong region of the brain. This would imply that the spiral tract does not belong to the auditory pathway. However, it is also possible that the spiral configuration is present in that region, and the experimental methods were unable to capture this configuration. Overall, qualitatively the white matter pathway modeled with DTI aligned well with the experimental structure (Fig.5).

Moving forward with this project will require scanning alligator brains with a higher resolution, obtaining the best suited tracking parameters for the auditory system, and finding a better way to place the ROIs in their correct location. Higher resolution scans will increase the sample size of this project and will also potentially make it easier to identify artifacts. Better tracking parameters will potentially better define the tracts, and also remove artifacts. Removing the user from placing the ROIs would be ideal to prevent user error. However, this may prove to be difficult given that not every DTI scan is alike.

This study demonstrated that DTI and tractography methods can be used to visualize the crocodilian auditory white matter pathway. This suggests that DTI is a viable method to visualize white matter pathways in the crocodilian brain. Being able to model white matter pathways in different species could potentially help us understand the evolutionary history of white matter pathways.

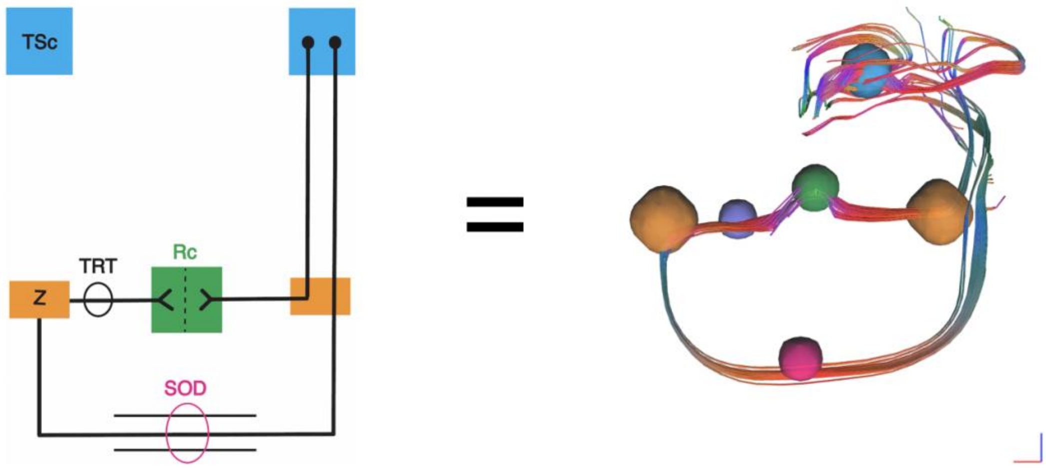

Figure 5: Comparison of the simplified experimental structure and DTI tractography results connecting the ROIs. Visually, the tracts using the DTI methods align closely with the tracts found using staining and histology.

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