Evaluating a Label-Free Method for Quantifying Cerebellar Changes in a Mouse Model of SCA8

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Introduction

In the past, in order to accurately visualize the mouse brain for quantitative measurement, time and resource intensive staining methods, such as Nissl or immunofluorescence, have been required (1,2). A newly developed label-free method using the Serial Optical Coherence Scanner (SOCS) has the potential to dramatically reduce the time required to prepare brain samples for analysis by utilizing the natural differences in the way white and grey matter scatter light to produce an accurate image from which numerous quantitative measurements can be taken (3,4,5). In order to establish that SOCS can be used for the same precision of analysis that can be performed using immunohistochemistry in measurements including cerebellar area/volume as well as differentiation and measurement of the main layers of the cerebellum, we utilized a mouse model predicted to have notable deficits in these areas.

A mouse model of Spinocerebellar ataxia type 8 (SCA8) has been documented to exhibit cerebellar Purkinje Cell death, which can be expected to thin the molecular layer, as well as demyelination, which would manifest in a decreased amount of cerebellar white matter (6,7). A profound cerebellar atrophy has been observed in multiple MRI studies of human SCA8 patients (8, 9). Together these instances of cell death and evidence from human patients should result in a measurable difference in area between SCA8 and wild type (WT) animals. In this small preliminary study, we wanted to determine if the SOCS method could be employed to parse out any difference in overall cerebellar area and molecular layer thickness between SCA8 and age matched WT mice.

Methods

- Age matched SCA8 and WT mice in late stages of disease progression were perfused with 4 % PFA
- Cerebellum was extracted and imaged sagittaly in 200 µm increments using the SOCS apparatus (3,5)
- Processed output provided retardance images for analysis

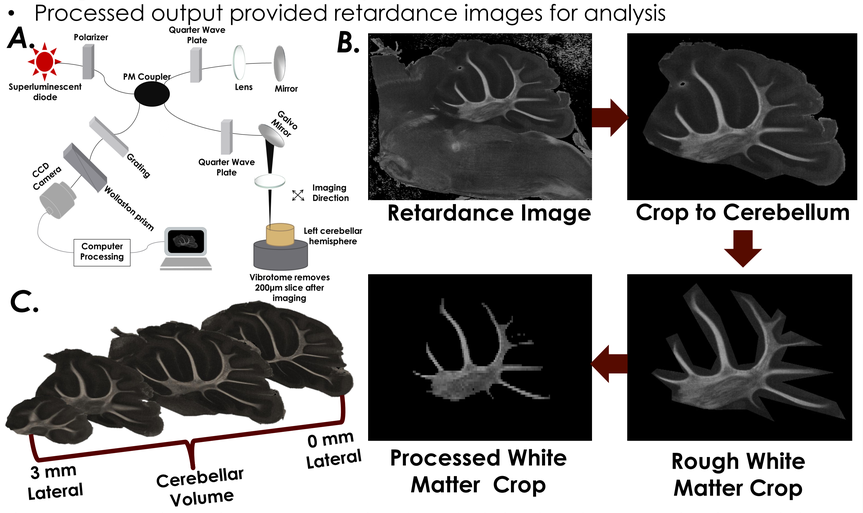

Figure 1: ***A.*** Experimental Apparatus. ***B.*** Flowchart depicting imaging processing for analysis. The retardance image, displaying the phase differences of the initially polarized x and y components of the light sample after they interact with the sample. The polarized light beam interacts in a significantly different manner with white and gray matter, allowing for a high contrast image for analysis. The brainstem was cropped out of the image in ImageJ, and a second image containing cropped white matter was processed in MATLAB to eliminate pixels with intensity below a set threshold to fully isolate the tract. This allowed for the cerebellar and white matter volume to be determined. ***C.*** Calculation of cerebellar volume. Values from slices located 0-3 mm lateral from midline were summed to determine overall volume. Each slice had a thickness of 200 µm.

Image Output

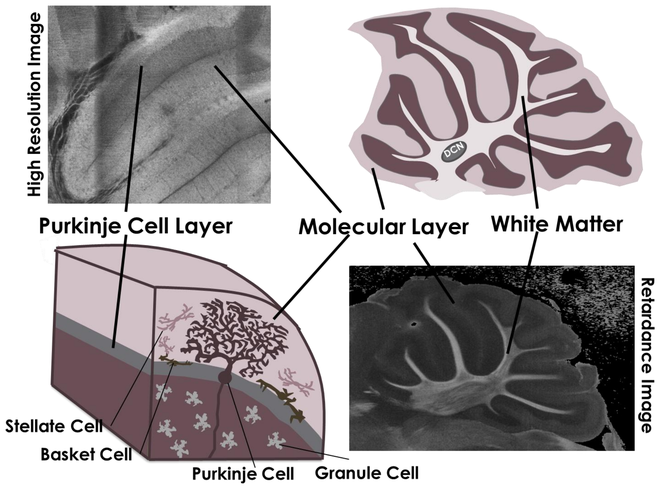

Figure 2: Diagram of relevant cerebellar layers and identification in retardance and high resolution (10x) output images. The molecular layer contains stellate and basket cells which synapse on the dendrites of Purkinje Cells also present in this layer. The layer of white matter contains axons of neurons involved in input and output systems of the cerebellum. The Purkinje Cell layer is readily identifiable in the high resolution retardance image and was used to measure the outer boundary of the molecular layer for measurements of molecular layer thickness.

Cerebellar Volume

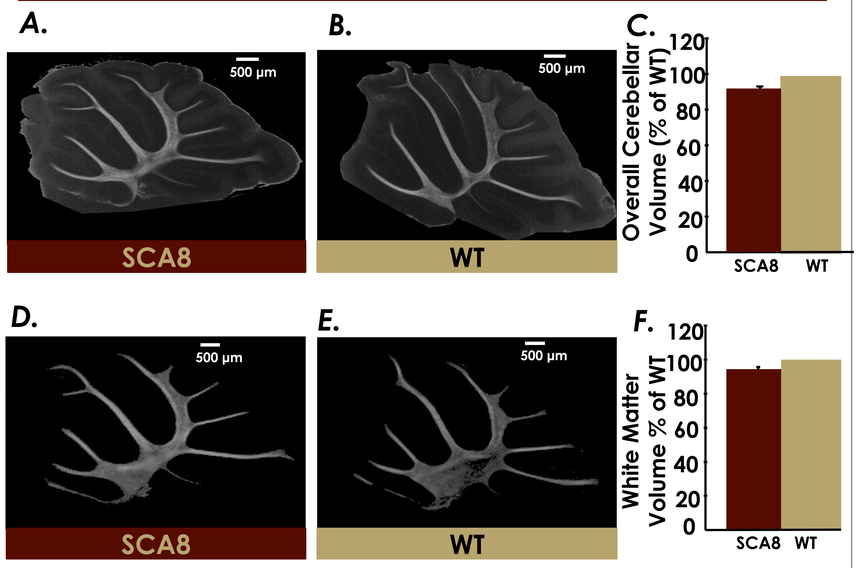

Figure 3: Cerebellar volume images and graphs. ***A***: Overall cerebellar volume, composite of white and gray matter. Example retardance SCA8 cerebellar slice from midline. ***B.*** Example retardance WT slice from midline. The cerebellar area of this slice is notably larger than the SCA8 slice. ***C.*** Graphical representation of the overall cerebellar volume located between 0 and 3 mm lateral from midline, standardized to the WT value. There is a trend towards a difference between the overall cerebellar volume of SCA8 and WT animals in the collected SOCS images. ***D.*** White matter volume measurements. Example retardance SCA8 cerebellar slice from 0.725 mm lateral from midline. ***E.*** Example retardance WT slice from midline. ***F.*** Graphical representation of the overall white matter volume located between 3 and 0 mm lateral from midline, standardized to the WT value. There is a notable difference between the overall white matter volume of SCA8 and WT animals in the collected SOCS image.

Molecular Layer Thickness

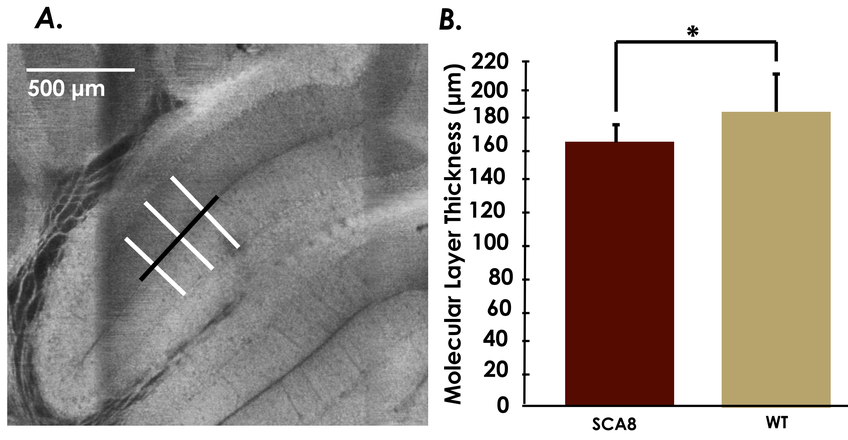

Figure 4: Molecular layer thickness measurements. ***A.*** Six measurements (white lines) of the molecular layers were taken directly adjacent to the primary folia (black line) (10). Three high resolution slices were imaged from the from the midline for each animal. In this image, the bodies of individual Purkinje Cells can be seen, and were used to measure the image boundary of the molecular layer. ***B.*** Average molecular layer thickness of SCA8 and WT cerebellum at midline. The average SCA8 molecular layer thickness,165.7 +/- 11.8 µm, was significantly lower than the average WT thickness, 185.9 +/- 24.5 µm (p=0.017). On average, the SCA8 molecular layer was 10.8% thinner than WT. WT measurements agreed with previously reported values (10).

Conclusion

- The Serial Optic Coherence Scanner (SOCS) is a useful label-free method for quantitative measurements of the overall volume of the white matter tracts and gross mouse brain, as well as the thickness of the molecular layer
- The SOCS apparatus is able to generate images with sufficient contrast to differentiate between the granule, Purkinje, molecular, and white matter cell layers of the cerebellum
- Quantitative analysis of SOCS images is able to yield predicted differences between WT and a SCA8 mouse model predicted to have notable cerebellar atrophy and a thinning of the molecular layer
- We are currently processing more samples to help establish cerebellar atrophy as a pathological characteristic of this mouse model of SCA8

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