ULTRASONIC ASSESSMENT OF WHITE MATTER AS A FUNCTION OF PREDOMINANT AXON ORIENTATION

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INTRODUCTION

The material properties of neuronal white matter are believed to vary depending on the orientation of the axons. Ultrasonic techniques provide a non-destructive method for the characterization of the material properties of tissues, which make them ideal for investigating brain. Acoustic microscopy can be used to measure the velocity of an ultrasonic wave, which can then be related to the tissue material properties. The objective of this study was to evaluate the variation in wave velocity and material properties for white matter as a function of axonal orientation.

METHODOLOGY

The specimen used for testing was fresh lamb brain, obtained locally. Each lamb's head was retrieved from the slaughterhouse and immediately returned to the lab. The skull was opened with a roto-zip saw, and the brain was removed intact from the cranium within 1 hour of death. The brain stem was then removed by blunt dissection and sliced to obtain either transverse or longitudinal sections. Given the well-defined orientation of axons in the brain stem (in the anatomical superoinferior direction), the two types of slices provided samples for which properties could be characterized either perpendicular to the axonal orientation (longitudinal) or along the axonal direction (transverse). Eight samples were obtained for each orientation from eight brains.

The tissue was weighed and the volume was measured in order to determine the density (ρ) of the sample. The specimen was sandwiched between plexiglas and aluminum plates, secured, and placed in a bath filled with artificial CSF (Sugawara, 1996) (Figure 1).

An ultrasonic scan was performed using a Sonix acoustic microscope at a frequency of 90 MHz. Three gates were set up to measure the time of flight of the pressure wave (t_1, t_2, and t_3) reflecting from the plexiglas, from the plexiglas/white matter interface, and from the white matter/aluminum interface. The acoustic microscope provides a greyscale image with time of flight graded on a linear scale from 0 to 255. This resulted in three graphical scans from which a correlation was made between grayscale and time of flight (Figure 2).

Appropriate calculations for wave velocities can then be made based on the knowledge of the thickness of the materials through which the wave passes.

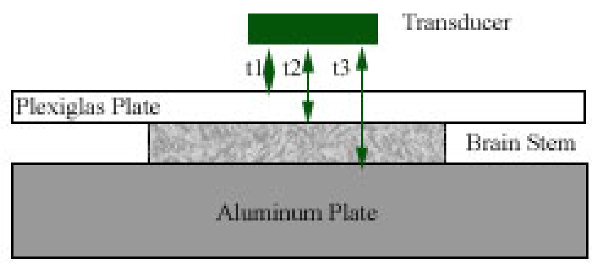

Figure 1 – Schematic diagram of acoustic microscopy measurement.

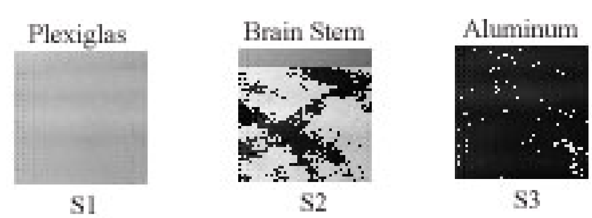

Figure 2 – Surface interface scans

The scans were analyzed utilizing Scion Image software to determine the mean and standard deviation of the time of flight (TOF) for each sample. The wave velocity (C_p) was calculated based on the thickness of the specimen and the time difference between the second and third reflected waves. A combination of bulk and shear moduli (K + 4G/3) was then calculated, using the measured wave speed and tissue density, from the equation:

$$C_P = [(K + 4G/3)/\rho]^{1/2}.$$

RESULTS AND DISCUSSION

The values calculated for wave velocity and combined modulus (K + 4G/3) are given in Table 1 for the transverse and longitudinal specimen orientations. There is a significant difference in both the modulus and velocity values between the two axonal orientations. When the ultrasonic wave traveled down the length of the axon in the longitudinal brain stem sections, the combined modulus was significantly higher than the values obtained for waves traveling perpendicular to the axon orientation. As the bulk modulus of a tissue is by definition independent of orientation, the variation in combined modulus can be used to estimate the differences in the shear moduli of the tissue depending on the orientation of the axons.

Sample Orientation	Sample Number	Wave Velocity (C_p) [m/s]	Combined Modulus (K + 4/3 G) [Mpa]
Longitudinal	1	1455	2433
	2	1487	2521
	3	1442	2422
	4	1477	2489
	5	1457	2464
	6	1481	2502
	7	1442	2404
	8	1446	2408
	Mean (SD)	1460 (18.24)	2455 (45.01)
Transverse	1	1318	1989
	2	1366	2102
	3	1333	2005
	4	1375	2118
	5	1355	2088
	6	1389	2269
	7	1396	2288
	8	1323	1996
	Mean (SD)	1356 (29.77)	2106 (117.20)

Table 1 – Results for brain stem in both longitudinal and transverse orientation

It should be noted that the shear modulus estimated for a transversely isotropic material using an applied pressure wave will be characteristic of shearing along the direction of wave propagation – not perpendicular to the wave front as would normally be expected. Thus, the data indicate a higher shear modulus for white matter for displacements along the axis of the axon (longitudinal samples) compared to shear displacements perpendicular to the axon axis (transverse samples).

SUMMARY

The resistance to shear is greater along the axis of the axon compared to perpendicular to the fiber. This confirms what would be expected based on the fibrous structure of the white matter. Acoustic microscopy allows for the characterization of material differences in small tissue specimens without the problems associated with traditional mechanical testing and at a much higher resolution. This technique should allow for future experiments that will provide additional insight into properties of these and other tissues.

REFERENCES

Osamu Sugawara, et. al. (1996). *Spine* **21**, 18, 2089-2094,.

ACKNOWLEDGMENTS

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