Transverse Relaxation Distributions of Mammalian Optic Nerve: An Approach to the Component/Compartment Relationship Using Glutamate

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Introduction: While MRI experiments provide anatomical and physiological information of tissue at a macroscopic scale, microscopic information at the cellular level can be accessed from multi-component T2 relaxation spectra. It has been shown that the rat optic nerve spectrum is characterized by three components [1] believed to arise from signal originating from the myelin, extra-axonal and axonal water. There is considerable evidence to assign the signal of the short-lived component to myelin water [2]. The interest of this study was to establish the relationship between the intermediate- and long-lived components to the water compartments from which they arise. This was accomplished through the induction of cell swelling caused by the neurotransmitter glutamate, reported to result in neuronal and glial cell swelling in the CNS [3, 4]. No reports have appeared for a corresponding swelling in the PNS. *In-vitro* experiments were performed using rat optic and sciatic nerves, the latter as a control, to access changes in their T2 relaxation spectra following incubation in a glutamate rich buffer solution. A better understanding of the component/compartment relationship of the T2 relaxation spectrum of CNS may give information that can be valuable in the diagnosis of pathologies.

Methods & Materials: Experiments were performed on a SMIS 3T NMR Scanner. Six Sprague-Dawley rats were euthanised and the optic and sciatic nerves excised for analysis *in vitro*. The nerves were placed in a perfusion chamber with a standard buffer solution flowing at a rate of 6.5 ml/hr or to which had been added glutamate (10 mM, Sigma Aldrich) to induce cell swelling. Data was acquired using a CPMG sequence with a TE of 1.6 ms, TR of 14 s, and 4000 echoes [5]. Data was analyzed using a non-negative least squares routine to determine the relaxation time components of the nerve. Ten or more spectra (each composed of 8 averages) of the nerves in the standard buffer solution were compared with > 20 spectrums in the glutamate solution. A paired *t-test* was performed in order to compare the results in the standard and glutamate buffer solutions.

Results: The use of a perfusion chamber allows recording data continuously and tracking changes in the relaxation times and relative sizes of the nerve components following addition of glutamate to the buffer solution. Figure 1 and Table 1 show data for the optic and sciatic nerves. Although the T2 times of both the optic and sciatic nerve components decreased following the addition of glutamate, those of the optic nerve exhibited a larger decrease. Following the introduction of glutamate the T2 time of the buffer solution decreased from approximately 1,450 ms to 1,090 ms (data not shown). However, while the component sizes of the sciatic nerve showed little or no change following the addition of glutamate, the long-lived component of the optic nerve exhibited a significant increase and the short-lived a significant decrease (Table 1a).

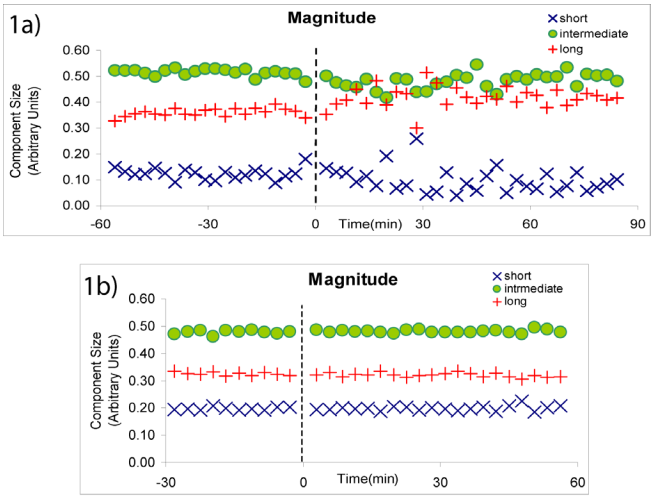

Fig. 1- Relative sizes of the three T2 spectral components of a) rat optic nerve and b) scitic nerve. At time zero the perfusing solution was switched from a standard buffer solution to one containing 10-mM glutamate.

Discussion: The primary finding of this study is that exposure of rat optic nerve to glutamate results in an increase in the size of the long-lived component of the T2 relaxation spectrum of the nerve. As glutamate has been reported to result in neuronal and glial cell swelling in the CNS, this suggests the long-lived component to result from intracellular water, either axonal and/or glial. That the component sizes require on the order of 30 min to stabilize in optic nerve following the introduction of glutamate (Figure 1a) is consistent with glutamate affecting the nerve and not the changes arising as an artifact of the introduction of glutamate to the buffer solution, as switching of the buffer solution in the flow chamber results in a purging of the previous solution to > 99% within < 4 min. Lack of any significant change in the component sizes of the sciatic nerve (Figure 1b) is consistent with glutamate resulting in a change within the optic nerve component sizes. The reduction in T2 relaxation times of the nerve components, especially those of optic nerve, is consistent with glutamate entering all nerve compartments as glutamate was observed to reduce the T2 relaxation time of the buffer solution. An incidental finding is that the introduction of glutamate reduces the short-lived component size associated with myelin water.

Conclusions: Based on the effect of glutamate it was determined that the long-lived component of the T2 relaxation spectrum of rat optic nerve appears to result from signal coming from the axonal and/or glial cell water. A smaller decrease in size of the intermediate-lived component is consistent with assignment of this component to signal coming from water in the extra-axonal space.

Table 1- Average values ± S.D. of spectral parameters for a) rat optic nerve and b) rat sciatic nerve incubated in a standard buffer solution and a 10-mM glutamate solution. P-values from a paired t-test of 10 sampled points in a standard buffer solution and 10 after 30 min of the addition of glutamate.

	a) Rat Optic Nerve (n=6)						b) Rat Sciatic Nerve (n=3)					
	Short		Intermediate		Long		Short		Intermediate		Long	
	T2(ms)	Size(%)	T2(ms)	Size(%)	T2(ms)	Size(%)	T2(ms)	Size(%)	T2(ms)	Size(%)	T2(ms)	Size(%)
Buffer	35±6	14.0±3.4	93±6	48.8±3.1	257±18	37.3±2.8	20±3	17.6±2.8	95±7	52.1±3.9	273±16	30.3±1.8
Glutamate	29±6	10.7±3.2	81±7	47.3±2.8	217±17	41.8±3.4	18±3	15.3±4.2	93±9	54.1±5.0	263±17	30.6±1.5
P-value	<0.001	< 0.001	< 0.001	0.008	< 0.001	<0.001	0.073	0.012	0.30	0.088	0.027	0.44

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