MRI Measurements of T2-Relaxation in a Rat Optic Nerve

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Abstract: Detailed Transverse relaxation time (T2) MRI imaging studies of a rat optic nerve model were performed in order to elucidate the effect of axonal regeneration in an optic nerve after traumatic injury. After rats were subjected to left optic nerve crush, a Carr-Purcell-Meiboom-Gill (CPMG) imaging sequence was used to acquire T2 relaxation decay curves from both the crushed left optic nerve (LOP) and the control right optic nerve (ROP) one week and one month after injury. The LOP and ROP T2 relaxation decay curves were best fit to biexponential functions, which were tentatively assigned to myelin associated water protons and water within or between axonal compartments. The fast T2 relaxation time and the fractional size in LOP one week after injury were significantly less than in ROP, indicating a reduction in myelin associated water and a change in its motional state. One month after injury, no significant differences in the fractional sizes and relaxation times in LOP and ROP were observed, consistent with nerve regeneration and repair of the crush injury.

Key words: biexponential, T2 relaxation, optic nerve.

INTRODUCTION

MRI has been used to study the organization and distribution of water in tissues. Previous in vitro studies of proton transverse relaxation of peripheral nerve revealed a multi-exponential decay characterized by at least three T2 components[1,2]. These components have been reported for in vitro (non-imaging) studies of amphibian (1), rat (2) and sciatic nerve. However, a recent in vivo study of rat trigeminal nerve revealed two T2 components with the short T2 component generally assigned to myelin associated water[3].

The origin of multiexponential transverse relaxation (T2) is generally accepted to be mainly due to a spatial compartmentation of water into intra- and extracellular spaces[4], although other interpretations have also been proposed[5,6]. In muscle for example, about 80 to 90 % of the signal can be attributed to the intracellular phase (spin-spin relaxation times of 20–40 ms), whereas the extracellular phase (spin-spin relaxation times of 100 ms or greater) contributes approximately 10 to 20 % of the overall signal[7]. For most quantitative MRI based T2 studies, there has been a tendency to assume monoexponential T2 relaxation and to employ the use of only a few echo times to measure single T2 relaxation times from tissue[8]. However, less technically complex but more clinically feasible CPMG imaging sequences with slice selective excitation and refocusing pulses and multi-slice capabilities have also proven capable of extracting biexponential T2 decay curves from some tissues[9,10]. The purpose of this study is to assess the potential of multiexponential T2 measurements by conventional CPMG sequence to elucidate the processes of axonal regeneration in an optic nerve after traumatic injury.

MATERIALS AND METHODS

Animal Preparation: Experiments were performed on young adult female Sprague Dawley rats (N = 8) weighing between 250-300 g. The rats were subjected to left optic nerve crush using standard techniques. Briefly, anesthetized adult female rats were fixed in a stereotaxic frame and an incision was made behind the left eye. The intra-orbital position of the left optic nerve was exposed and then crushed 2 mm behind the globe for 10 seconds using a small forceps. Nerve injury was verified by the appearance of a cleaning at the crushed site. Prior to the imaging course all the rats were anesthetized with 2 % isoflurane (Forane £, Baxter Healthcare Corporation, Deerfield, IL). After the end of each experiment rats were sacrificed with an overdose of pentobarbital (100 mg/kg). All procedures were approved by the Harvard Medical School Animal Care and Use Committee protocols (Boston, MA).

MRI Measurements: Experiments were performed using a volume coil on a Bruker Biospec (Bruker Instrumentation, Inc., Billerica, MA) operating at 4.7 T.

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with a maximum gradient strength of 400 mT/m. After matching, tuning of the coil, shimming and pulse calibrations, the rat brains were imaged with a multi-slice T2 weighted sequence. From the T2-weighted images a single slice in which both the right and left optic nerves were delineated was selected and imaged at one week and one month after the surgery.

**T2 Relaxation Measurements:** A manufacturer supplied CPMG imaging sequence with slice selective excitation and refocusing pulses and phase encode reinders\(^{[9,10]}\) was applied to acquire 18 echoes with an 6.68 ms echo spacing (TE) from the selected slice. Other imaging parameters were: repletion time TR = 3 sec, slice thickness = 1 mm, in-plane image matrix = 128 x 128, FOV of 3 x 3 cm\(^2\) and NEX =1. The slice selective 90° and 180° RF pulses were 3 lobe sinc pulses with pulse durations of 2000 µsec and bandwidths of 2250 Hz.

For each rat, T2 decay curves consisting of signal versus echo time were extracted from the LOP and the ROP. The background noise was also measured from an air containing ROI along the readout direction. The resulting signal decay curves from the selected regions were then found to be best fit with biexponential functions of the form:

\[
S = A_f \exp\left(-n2\tau/T_{2f}\right) + A_s \exp\left(-n2\tau/T_{2s}\right)
\]

Where \(A_f\) and \(A_s\) are the amplitudes of the fast and slow relaxation components with relaxation times \(T_{2f}\) and \(T_{2s}\), respectively; \(2\tau\) is the echo spacing (6.68 ms); and \(n\) is an integer ranging from 1 to 18. All fits of the data to biexponential functions were performed with a Levenberg-Marquardt non-linear least square algorithm\(^{[11]}\) as implemented in Matlab software (The MathWorks Inc., Natick, MA).

**RESULTS AND DISCUSSION**

Fig. 1 shows the 3rd echo image from 18 echo slice selective CPMG imaging sequence of an *in vivo* rat brain. The image shows the ROI’s in the optic nerves in crushed and contralateral sides one week after the injury. Fig. 2 shows typical semi-log plot of the T2 relaxation data from a ROP and a LOP ROI, as well as baseline noise values extracted from an air containing ROI one week after the injury. Solid lines through the LOP and ROP decay curves are best fits to biexponential functions. There is a distinctly larger signal from LOP at the later echo times indicating a more pronounced component with a longer relaxation time than found in the healthy ROP one week after injury.

**Fig. 1:** The 3rd echo image of the CPMG imaging sequence of an *in vivo* rat brain showing the LOP and the ROP.

**Fig. 2:** T2- decay curves from LOP, ROP and noise 1 week after injury.

**Fig. 3:** T2- decay curves from LOP, ROP and noise 1 month after injury.

Typical relaxation decay curves one month after injury for ROP and LOP ROIs are provided in Fig. 3 along with baseline noise values. Bi-exponential fits to the
consistently with an interpretation that the crush injury following nerve injury and regeneration. Our results are of the biophysical state of water within them following nerve injury and regeneration. Our results are consistent with an interpretation that the crush injury squeezes water associated myelin out, reducing the observed fast relaxing fraction and that furthermore, water within that compartment has substantially more freedom of movement than in the healthy state, leading to a longer “fast” relaxation time. The return of the damaged decay curve to water fractions and relaxation times similar to the healthy nerve after one month is, we believe, a sign of regeneration and repair such that the normal water balance and state of water within the two compartments has been achieved at one month following this particular injury.

Table 1: Changes in short and long component of T2 relaxation time the amplitudes of the fast and slow relaxation components in the optic nerves in crushed and contralateral sides 1 week and 1 month after surgery.

<table>
<thead>
<tr>
<th></th>
<th>LOP</th>
<th>ROP</th>
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<tbody>
<tr>
<td>T2f (ms)</td>
<td>205±4.62</td>
<td>201±4.3</td>
</tr>
<tr>
<td>T2s (ms)</td>
<td>22.1±3.2</td>
<td>32.8±3.2</td>
</tr>
<tr>
<td>A/(A+S+Af)</td>
<td>0.31±0.04</td>
<td>0.21±0.02</td>
</tr>
<tr>
<td>Af/(A+S+Af)</td>
<td>0.69±0.04</td>
<td>0.79±0.02</td>
</tr>
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A, and A, are the amplitudes of the fast and slow relaxation components with relaxation times T and T.

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REFERENCES
