MAGNETIC RESONANCE OF HUMAN AND BOVINE BRAIN

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Abstract

Magnetic resonance imaging (MRI) has become an invaluable tool for studying brain and its associated pathologies. Multiple sclerosis (MS) is one such pathology and attempts are being made to use MRI to characterise the myelination state of MS lesions.

Two techniques have been proposed which appear to be sensitive to myelination: magnetization transfer (MT) and T2 relaxation. Quantification of these techniques uses magnetization transfer ratios (MTR) for MT and myelin water percentages for T2 relaxation. If the two techniques are both related to myelin content then they are expected to be related to each other. It was found by in vivo MRI measurements that white matter from normal volunteers and normal appearing white matter from MS patients had significantly larger MTRs and myelin water percentages than grey matter. However, only a weak correlation was found between MTRs and myelin water percentages in MS lesions (\(R=0.5, P=0.005\)) indicating that each technique provides an independent measure of MS pathology.

Since water in white matter resides in two main compartments, in intra/extracellular spaces and between myelin bilayers, it was thought that MT would have a different effect on each water pool. This was examined by combining a T2 relaxation sequence, which separates the two water pools, with an MT pulse. It was found using in vivo MRI measurements on normal human white matter that the myelin water pool was significantly more affected by an MT pulse than the intra/extracellular water pool (\(P=0.00001\) to \(P=0.04\) for different white matter structures). It was also found that small offset frequencies caused more direct saturation of the myelin water pool than the intra/extracellular
pool resulting in different contrast. Finally, at long delay times between the MT pulse and the initiation of the $T_2$ relaxation sequence (>500 ms), the difference in MT between the two pools was eliminated indicating exchange within that timescale.

*In vitro* experiments on bovine brain were performed on a $^1$H-NMR spectrometer. A 4-pool model was proposed to explain the different relaxation times measured in bovine white matter. These pools included intra/extracellular water, myelin water, non-myelin molecules and myelin molecules. Exchange between the myelin water and myelin, and the intra/extracellular water and non-myelin molecules were rapid with the former being slightly faster than the latter. There was no evidence for exchange between the two water pools within the timescale of 1 s.

For human brain, a diffusion model was proposed to investigate exchange between the water pools. Results showed that variations in parameters associated with the intra/extracellular water pool affected only that pool. Variations in the myelin water pool, however, influenced the relaxation times and amplitudes of both water pools. Finally, it was found that changes in the axonal diameter and myelin thickness resulted in changes in the myelin water percentages and $T_2$ relaxation times. This could account for some of the differences in myelin water percentages and $T_2$ times measured in different white matter structures in the human brain.
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Chapter 1

Introduction

1.1 Brain and Myelin

The brain is made up of neurons and glial cells which work together to carry out brain function. The neurons form the communication network in the body while the glial cells act as a support structure. A sketch of a typical nerve cell is shown in Figure 1.1. The nerve cell is made up of four different structures, the cell body (which is the metabolic centre of the cell), the axon (which transmits nerve impulses also known as action potentials over long distances), the dendrites (which receive signals from other neurons) and the pre-synaptic terminal (which releases neurotransmitter in response to action potential in order to pass on information to the next neuron). Axons can propagate for distances up to 1 m and range from 0.2-20 µm in diameter. The axon connects to the cell body at a region called the action hillock. At this location, the composition of the neuron is unique in order to allow the initiation of action potentials via an integration of all signals received by the cell. In order for signals to propagate quickly along the length of the axon, an insulating structure called the myelin sheath is present. Myelin is described in more detail below. At its terminus, the axon divides into small branches each with a pre-synaptic terminal. These terminals end in proximity to other dendrites or cell bodies in order to transmit the signal, through release of neurotransmitters, to the next cell. Therefore, signal propagation in a neuron begins with neurotransmitters binding to specialised receptors in the dendrites or cell body where these chemical signals
are transformed into electrical signals. These electrical signals then transmit passively to the axon hillock where they are integrated and, if a certain threshold is reached, an action potential is generated. This action potential flows uni-directionally down the length of the axon until it reaches the pre-synaptic terminals. Modulated by the number and frequency of the action potentials, neurotransmitter is released from the pre-synaptic terminal. The neurotransmitter then diffuses to the next cell and binds to receptors. The whole process begins again. Depending on the type of neuron being fired, an inhibitory or excitatory signal can be delivered to the post-synaptic cell.

Figure 1.1: A sketch of a typical nerve cell in the brain. From left to right are the pre-synaptic terminals, the myelinated axon, the cell body and the dendrites. [7]

The myelin sheath is a lipid-protein membrane found in the central nervous system (CNS) and peripheral nervous system (PNS) of vertebrates [1, 2]. In the CNS, it is created by specialised glial cells called oligodendrocytes which wind themselves tightly around the axon (Figure 1.2) [3, 4]. The resulting multilayer is composed of repeating units of membrane-cytoplasmic space-membrane-extracellular space with a thickness of 150-160 Å[5]. Dehydrated myelin is composed of 75-80% lipid and 20-25% protein [6]. This is quite unusual since other membranes are generally 50% lipid and 50% protein.
Myelin makes up 50% of the dry weight of white matter and has a relatively low water content of 40% [6]. The myelin is present to act as an electrical insulator for neurons and allow conduction of nerve signals to propagate about 100× faster. There are gaps along the axon where no myelin is present known as the nodes of Ranvier which are important in signal conduction.

Figure 1.2: An electron micrograph of myelin. The bar represents $0.1\mu m \times 150000$. [1]

Since nerve impulses consist of electrical signals, it is important to know how these are created and how they propagate. A nerve cell membrane has a membrane resting potential of -65 mV. (The negative sign indicates that the inside of the cell is negatively
charged with respect to the outside of the cell.) This potential is due to the uneven distribution of ions, namely Na\(^+\), K\(^+\), Cl\(^-\) and Ca\(^{2+}\), across the membrane. In signal transduction, the two most important ions are Na\(^+\) and K\(^+\). The Na\(^+\) concentration is about 10 times lower inside the cell than outside while the K\(^+\) concentration is about 50 times higher inside than outside [7]. These gradients are maintained by a Na\(^+\)/K\(^+\) pump and also by the leakiness of the plasma membrane to K\(^+\) but not Na\(^+\). In the membrane, there are voltage gated ion channels which only open when the membrane becomes depolarised. The interplay between these channels creates the action potential which propagates along the axon (shown in Figure 1.3). When the membrane potential is increased by about 10 mV (from -65 to -55), an action potential is fired. The initial depolarisation causes voltage gated Na\(^+\) channels to open and Na\(^+\) rushes into the cell. This causes a large depolarisation to about +110 mV. The Na\(^+\) channels have a self-regulating feature which automatically closes them after a short time. At this time, voltage gated K\(^+\) channels open and K\(^+\) rushes out of the cell causing the membrane to become repolarised. This efflux of K\(^+\) actually overshoots the initial resting potential and the membrane becomes hyperpolarised. The K\(^+\) channels close and the Na\(^+\)/K\(^+\) pump restores the ion gradients. The overall duration of an action potential is about 1 ms. Cl\(^-\) is often passively distributed across the membrane although there are sometimes pumps which pump the Cl\(^-\) out of the cell. This ion will also help contribute to the action potential but to a lesser extent. As the membrane becomes depolarised, it dissipates quickly along the axon. This passive conduction of the depolarisation can cause neighbouring regions of the axon to reach the threshold and a new action potential is triggered. If myelin is present, the rate of dissipation of the depolarisation is much slower. At the nodes of Ranvier, Na\(^+\) channels are found in large concentrations making the triggering of action potentials very easy. Therefore, the action potential can hop along the axon from node to node which allows it to travel much faster. This propagation of action
potentials is called saltatory conduction.

Figure 1.3: A sketch of an action potential. The two solid lines show the flux of Na\(^+\) and K\(^+\) through ion channels and the dashed line represents the resulting action potential. The horizontal reference line is at -65 mV and the top of the action potential reaches about +110 mV. [7]

1.2 Multiple Sclerosis

In Canada, the number of people afflicted with multiple sclerosis (MS) is one of the highest in the world. It is thought that over 50000 Canadians have the disease. MS is usually diagnosed at an early age, between 20 and 40 years and the ratio of women to men affected is 1.8:1. MS is characterised by the destruction of CNS myelin. The disease is thought to be brought on by both environmental and genetic factors. A virus is thought
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to play a role but none has been definitively linked \([8, 9]\). There are two forms of MS: a chronic form (90% of cases) which manifests as a continuous relapsing/remitting cycle of demyelination and an acute form (10% of cases) where there is rapid and progressive demyelination. The chronic form usually consists of relapses and then subsequent recovery of most disabilities. After several years, complete recovery no longer occurs and the disease becomes known as secondary progressive. Demyelination arises from an autoimmune response targeting myelin antigens which are thought to derive from myelin basic protein or some other myelin specific protein \([1]\). These areas of demyelination are referred to as lesions. Early lesions have been correlated with the breakdown of the blood brain barrier near small vessels. The lesions are then believed to become inflamed followed by demyelination, gliosis and finally axonal loss. Lesions can be visualised by computerised tomography (CT) and magnetic resonance imaging (MRI) \([10, 11, 12]\) although the images are generally non-specific to the state of the lesion. MS lesions become infiltrated by immune cells such as macrophages, neutrophils and lymphocytes. The macrophages appear to destroy myelin sheaths by digesting the outer layers until all myelin is gone. Strangely, this cellular attack will stop at a boundary and the white matter beyond looks relatively normal. There is no known cause for this behaviour. Normally the demyelinated tissue would be repaired but the disease also affects the body's ability to replenish the myelin. Oligodendrocytes do attempt to remyelinate some neurons but they are both slow and inefficient. Although lesions are the main locations for disease, there is evidence that normal appearing white matter may also have small abnormalities which are difficult to see with MRI (hence the term normal appearing). These abnormalities may add to the overall lesion load and contribute to disabilities acquired by the patient.
1.3 Motivation

Myelin is found wrapped around axons in brain white matter and helps speed up nerve signal conduction. Unfortunately in certain diseases such as multiple sclerosis (MS), myelin breaks down. Magnetic resonance imaging (MRI) is a fairly new technique which has proven useful in detecting MS lesions. However, MRI is not capable of detecting the pathological state of the lesion and correlation between lesion load and disability has been small [13, 14, 15]. Therefore efforts are being made to produce an MRI sequence which will be able to probe the state of the lesion and be able to quantify the amount of myelin.

Dr. Alex MacKay has developed an *in vivo* T$_2$ relaxation pulse sequence which allows the separation of different water pools in the brain. One of these pools is thought to be associated with myelin. Initial experiments on brain [16, 17] showed that white matter had larger quantities of this “myelin water” than grey matter. Also, MS lesions were found to have greatly reduced amounts of myelin water compared to the surrounding tissue. These experiments were very exciting since they showed that one component from T$_2$ relaxation seemed to be related to water associated with myelin which may be related to myelin content.

Elsewhere, the group of Dousset *et al.* began applying the technique of magnetization transfer (MT) to lesions [18]. Their initial findings showed that mainly edematous lesions produced a different MT effect than demyelinated lesions. These results indicated that MT might also be able to differentiate between different types of lesions.

Neither technique has fully validated its ability to measure myelin. A study was carried out where an MS-like disease (experimental allergic encephalomyelitis or EAE) was induced in primates and MT was subsequently performed [19]. From histological data, lesions with more demyelination were indeed found to have a larger change in
MT than non-demyelinated lesions. \( T_2 \) relaxation has been performed on guinea pigs with EAE [20] and histological samples showed that the lesions were demyelinated which correlated well with their reduced myelin water peak. Therefore, there is evidence that both these techniques are influenced by myelin content.

Since both techniques are thought to be related to myelination, we decided to compare them. If they are indeed proportional to the amount of myelin, then a linear correlation between them would be expected. This study was done on normal volunteers and MS patients. It was hoped that we could determine which technique was better at quantifying myelin.

From the results of \( T_2 \) relaxation, it was evident that there were different water pools in the brain. These water pools have different interactions with the macromolecules and therefore each pool should have a different MT effect. The \( T_2 \) relaxation pulse sequence was combined with the MT sequence in order to monitor the effects of MT on each water pool separately. Since most groups assume that there is only one water pool in the brain, this work would improve modelling in this area and also help in understanding the mechanisms involved with MT in the brain.

*In vitro* \(^1\text{H}\)-NMR experiments were performed to measure interactions between the different water pools as well as the surrounding macromolecules in order to provide a better understanding of the mechanisms of relaxation present in brain tissue. This in turn could lead to improvements in MR pulse sequences which could provide novel information in the diagnosis of different diseases.

Finally, a model for relaxation was proposed that involved diffusion between the two water pools. Numerical simulations were carried out to try and determine the effect of different parameters (such as diffusion coefficients and cell size) on the relaxation amplitude and time of each pool.
1.4 Review of NMR and MRI work on Brain

1.4.1 T\textsubscript{2} Relaxation

The T\textsubscript{2} relaxation decay curve from brain arises from all the water in the brain [20, 21]. Previous studies [16, 17, 22, 23] have shown that three water compartments may be distinguished on the basis of T\textsubscript{2} time: a long T\textsubscript{2} component assigned to cerebrospinal fluid, an intermediate T\textsubscript{2} component assigned to extracellular water and cytoplasm, and a short T\textsubscript{2} component assigned to myelin water (Figure 1.4). The myelin water is thought to be trapped between myelin bilayers where the short T\textsubscript{2} time derives from interactions between the water and the molecules in the myelin bilayers. The amount of water between myelin bilayers is expected to be proportional to the amount of myelin. In general, we ignore CSF which does not contribute much to the signal from white and grey matter so that there are two water pools in normal CNS tissue and they are resolvable on the basis of their T\textsubscript{2} relaxation.

Measurements of T\textsubscript{2} relaxation times in brain are not always done rigorously which leads to conflicting results in the literature [24]. T\textsubscript{2} relaxation pulse sequences must include more than 4 echoes and have echo spacings of 10 ms or less in order to measure both the intra/extracellular and myelin water. In white matter, the intra/extracellular water T\textsubscript{2} varies for the different structures (e.g. 86 ms for the internal capsules and 71 ms for the minor forceps) which is likely due to differences in myelin content [17]. In certain cases, the T\textsubscript{2} distribution for white matter shows two peaks in the 80–100 ms region which may be separation of the intracellular and extracellular water pools. The T\textsubscript{2} of intra/extracellular water in grey matter is also found to be of the order of 70–90 ms.

Some groups induced cerebral edema in animals in order to determine the effect on T\textsubscript{1} and T\textsubscript{2} relaxation [25, 26, 27]. When edema was induced, the longer T\textsubscript{2} component
Figure 1.4: A $T_2$ distribution of white matter showing the different water components in the brain. The area under each peak is proportional to the number of protons in that environment.
split into two peaks representing intracellular and extracellular water. The extracellular water peak was larger and at longer $T_2$ which was attributed to the extra water present from edema. The intracellular peak remained largely unaffected by the edema. Another experiment on cats studied the effect of gliosis [28]. $T_2$ times were found to be largely unchanged even in the presence of edema as well as white matter packed with glial fibrils and other structures. This lack of change in $T_2$ was attributed to the efficient cross relaxation between the water and the extra cytoplasmic structures resulting in no net $T_2$ change. Finally, in animals induced with EAE, edema in lesions caused the intra/extracellular water $T_2$ to increase [29, 30, 31].

In multiple sclerosis, the $T_2$ of normal appearing white matter was found to be slightly elevated compared to normal controls [32]. In lesions, large variations in $T_2$ were found which were thought to be due to different underlying tissue composition [33, 34, 35, 36]. In some cases, the large $T_2$ component had split into two peaks and was thought to represent axonal loss leading to a larger extracellular space [37, 38]. However, other studies have not been able to confirm this result [39, 40].

### 1.4.2 $T_1$ Relaxation

Unlike $T_2$ relaxation which is able to differentiate different water pools, $T_1$ relaxation of human brain appears to yield only one relaxation time. In a myelinated crayfish nerve, the $T_1$ was found to be about 1.2 s [22] which is slightly less than that expected for pure water. In the squid giant axon, a similar $T_1$ of 1.5 s was found [41]. In human brain however, the $T_1$ of white matter was about 600 ms whereas the $T_1$ of grey matter was 1 s [42]. Again, only mono-exponential relaxation was found. The difference in $T_1$ between white and grey matter is thought to be due to myelin and in particular interactions between myelin water and myelin molecules [21, 43, 44]. Supporting this theory are experiments measuring $T_1$ from newborns and adolescents which showed $T_1$ times of 1.6
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1.2 

The T1 relaxation times were linearly correlated with water content in brain tumour samples [46]. With edema, the T1 of white matter was found to increase more rapidly than grey matter. The suggested reason is that excess water in grey matter is taken up by cells where the T1 is reduced by the proteins present in the cytoplasm. In white matter, the excess water remains in the extracellular spaces which is free of such molecules. In animal models of edema, T1 was also increased [25, 26, 27, 29, 30, 31]. In gliosis, T1 was increased as opposed to T2 which did not change resulting in a decrease of T2 relative to T1 [28].

In MS patients, T1 increased in lesions but was still mono-exponential and therefore probably not as useful as multi-exponential T2 measurements [34, 35, 36]. There were however, large variations in T1 between lesions attributed to different underlying tissue structure. NAWM was also found to have an increased T1 [32].

1.4.3 Magnetization Transfer

Magnetization transfer (MT) is a relatively new MR technique which provides a novel form of contrast. It was developed by Wolff and Balaban [47] and based on a technique discovered by Forsen and Hoffman [48]. MT has become widely used to study many diseases, in particular multiple sclerosis [16, 18, 47, 49, 50, 51, 52, 53, 54, 55, 56]. MT [47] utilises the fact that there is continuous magnetization exchange between two proton pools in the brain: the motionally restricted pool which arises from non-aqueous tissue and the mobile pool from water [21]. MRI can only directly detect signal from the mobile pool. If the magnetization from the motionally restricted pool is disturbed by an MT pulse, then the effect of exchange can be seen on the mobile pool as a decrease in signal [48]. This effect is called magnetization transfer and is quantified through a magnetization transfer ratio (MTR) (further defined in Chapter 2). It is expected that
brain volumes which have a larger number of motionally restricted protons will show a
greater signal decrease upon application of an MT pulse and therefore a greater MTR.
Protons associated with myelin would be part of the motionally restricted pool. In this
way, the state of myelination of a lesion can be probed using MT.

1.5 Overview of Thesis

First, the groundwork will be set by reviewing some general theory in Chapter 2. This will
be followed by the general materials and methods used in all experiments (Chapter 3).
Further details on the materials and methods is given in the individual results chapters.
Three different studies are presented in the next three chapters. Chapter 4 compares
MTRs and myelin water percentages in normal volunteers and MS patients. Chapter 5
extends the work by incorporating both MT and T2 relaxation into one sequence and
studying the effect on normal volunteers. In Chapter 6, in vitro 1H-NMR studies are
presented from bovine brain. Many different experiments were carried out in order to
determine how different proton pools interacted and over what timescale. Simulations
of T2 and T1 relaxation are presented in Chapter 7 for human brain by assuming that
diffusion occurs between the different water pools. Finally in Chapter 8, conclusions
are made about how all the studies relate to each other. Also, future experiments are
suggested as well as some that are already underway.
Chapter 2

General Theory

2.1 Relaxation

$^1$H nuclei are spin 1/2 particles and possess magnetic moment $\mu$ and angular momentum $J$. These two quantities are related by

$$\mu = \gamma J$$ (2.1)

where $\gamma$ is known as the gyromagnetic ratio. The sum of all the magnetic moments in a sample gives the total magnetization denoted by $M$. If a magnetic field, $B$, is applied to the system, a torque results such that

$$\frac{dM}{dt} = \gamma (M \times B).$$ (2.2)

This equation produces precession of the magnetization around the external magnetic field at a rate known as the Larmor frequency given by

$$\omega_0 = \gamma B_0.$$ (2.3)

If a radio frequency (rf) pulse, $B_1$, is applied at this frequency, the energy will be absorbed and the net magnetization will tip by an angle $\theta = \gamma B_1 t_p$ where $t_p$ is the length of time that the rf field is applied.

When the magnetization is perturbed from its equilibrium state, it tries to return via a process called relaxation. One form of relaxation, denoted $T_1$, brings the magnetization back to equilibrium along the direction of the external magnetic field (normally assigned
Chapter 2. General Theory

to the z-axis). The other, denoted $T_2$, destroys any net magnetization in a plane perpendicular to the external field (x-y plane). These terms are added to equation 2.2 in order to obtain the Bloch equation

$$\frac{dM}{dt} = \gamma (M \times B) + \frac{M_o - M_z \hat{z}}{T_1} - \frac{M_x \hat{x}}{T_2} - \frac{M_y \hat{y}}{T_2}$$

(2.4)

where $M_o$ is the net magnetization at equilibrium.

For spin 1/2 particles, the most important relaxation mechanism is the dipole-dipole interaction. The dipolar Hamiltonian is given by

$$H_{12} = \frac{\gamma_1 \gamma_2 \hbar^2}{r^3} [I_1 \cdot I_2 - 3(I_1 \cdot \hat{n})(I_2 \cdot \hat{n})]$$

(2.5)

where $I$ is the angular momentum, $r$ is the separation between the two spins and $\hat{n}$ is the unit vector in the direction joining them. If we transform $\hat{n}$ into polar coordinates with angle $\theta$ and $\phi$ with respect to the external magnetic field, the Hamiltonian can be written as

$$H_{12} = \frac{\gamma_1 \gamma_2 \hbar^2}{r^3} (A + B + C + D + E + F)$$

(2.6)

where

$$A = I_1 z I_2 z (1 - 3 \cos^2 \theta)$$

$$B = -\frac{1}{4}(I_1^+ I_2^- + I_1^- I_2^+) (1 - 3 \cos^2 \theta)$$

$$C = -\frac{3}{2}(I_1^+ I_2 z + I_1 z I_2^+) \sin \theta \cos \theta e^{-i\phi}$$

$$D = -\frac{3}{2}(I_1^- I_2 z + I_1 z I_2^-) \sin \theta \cos \theta e^{i\phi}$$

$$E = -\frac{3}{4}(I_1^+ I_2^+) \sin^2 \theta \cos^2 2\phi$$

$$F = -\frac{3}{4}(I_1^- I_2^-) \sin^2 \theta \cos^2 2\phi.$$  

(2.7)

The terms $A$ and $B$ only allow for transitions between equal Zeeman energy states, the $C$ and $D$ terms allow one of the spins to flip (with energy transition of $\hbar \omega_o$) and the $E$
and F terms allow both the spins to flip (with energy transition of $2\hbar\omega_0$). Consequently, $T_1$ relaxation (which involves a net change in energy of the system) only results from the last 4 terms. $T_2$ relaxation arises from all terms.

If motion (in the limit of $M_2\tau_c^2 \ll 1$, where $M_2$ is the second moment of the dipolar broadened lineshape and $\tau_c$ is the correlation time for the motion) is present, then the dipolar interaction becomes averaged. In this case, relaxation can be described by Redfield's Theory [57]. For spins at a constant separation $r$ and varying in orientation isotropically, relaxation can be defined as

$$\frac{1}{T_1} = \frac{\gamma^4\hbar^2}{4r^6} [J(\omega_0) + 4J(2\omega_0)] \quad (2.8)$$

$$\frac{1}{T_2} = \frac{\gamma^4\hbar^2}{16r^6} [6J(0) + 10J(\omega_0) + 4J(2\omega_0)] \quad (2.9)$$

where $J(\omega)$ is the spectral density and equal to the Fourier transform of the correlation function. Usually, the correlation function is assumed to be exponential with a time constant $\tau_c$ so equation 2.8 and 2.9 become

$$\frac{1}{T_1} = \frac{3}{10} \frac{\gamma^4\hbar^2}{r^6} \tau_c \left[ \frac{1}{1 + \omega_0^2\tau_c^2} + \frac{4}{1 + 4\omega_0^2\tau_c^2} \right] \quad (2.10)$$

$$\frac{1}{T_2} = \frac{3}{20} \frac{\gamma^4\hbar^2}{r^6} \tau_c \left[ 3 + \frac{5}{1 + \omega_0^2\tau_c^2} + \frac{2}{1 + 4\omega_0^2\tau_c^2} \right]. \quad (2.11)$$

For short correlation times ($\omega_0\tau_c \ll 1$), one has

$$\frac{1}{T_1} = \frac{1}{T_2} = M_2\tau_c. \quad (2.12)$$

In bulk water, $\tau_c \sim 10^{-12}$s and $T_1$ and $T_2$ are between 1 and 3 s. In general for tissue, $T_2 < T_1$ due to interaction between water and macromolecules. For brain tissue, we expect more than one stochastic process to influence the relaxation and therefore equations 2.8 and 2.9 would involve more than one $\tau_c$. This would lead to much more complicated expressions.
2.2 Second Moment

The moments of a lineshape function $f(\omega)$ can be defined as

$$M_n = \frac{\int_0^\infty \omega^n f(\omega) d\omega}{\int_0^\infty f(\omega) d\omega} \quad (2.13)$$

The so-called second moment comes from $n=2$.

For a rigid lattice, the second moment can be calculated from

$$M_2 = \frac{3}{4} \gamma^4 \hbar^2 I(I+1) \sum_k \frac{(1-3 \cos^2 \theta_{jk})^2}{r_{jk}^6} \quad (2.14)$$

where $r_{jk}$ is a vector describing the relative position of two protons and $\theta_{jk}$ is the angle between the applied magnetic field and this vector. If one averages over all angles, equation 2.14 reduces to

$$M_2 = \frac{3}{5} \gamma^4 \hbar^2 I(I+1) \sum_k \frac{1}{r_{jk}^6} \quad (2.15)$$

In the presence of motion, the lineshape becomes narrowed and the measured second moment will be smaller. (The true second moment never changes.) This is known as motional narrowing.

Unfortunately, equation 2.15 is very hard to apply experimentally. Instead, the Lowe-Norberg theorem relates $f(\omega)$ to a free induction decay function $F(t)$

$$F(t) = \int_0^\infty f(\omega) \cos(\omega t) d\omega \quad (2.16)$$

where $\int_0^\infty f(\omega) d\omega = 1$ is assumed. If $\cos(\omega t)$ is replaced by its Taylor's series, then we get

$$F(t) = \sum_0^\infty (-1)^n \frac{t^{2n}}{(2n)!} M_{2n} \quad (2.17)$$

Therefore, a free induction decay can be used in order to determine the second moment.
2.3 Cross Relaxation and Exchange

Cross relaxation can be represented by a modification of the Bloch equations to incorporate exchange of magnetization between two pools of protons (A and B) [58]. The longitudinal magnetization in pool A is designated \( M_A \) and in pool B \( M_B \). The fully relaxed magnetizations are designated \( M_{A0} \) and \( M_{B0} \). The rate constant for exchange from pool A to pool B is \( K_A \) and from pool B to pool A is \( K_B \). The reciprocal of the rate constants give the lifetimes that protons spend in each pool, i.e. \( K_A = \frac{1}{\tau_A} \) and \( K_B = \frac{1}{\tau_B} \).

When exchange occurs, pool A loses \( \frac{M_A}{\tau_A} \) magnetization to pool B per unit time and pool B loses \( \frac{M_B}{\tau_B} \) magnetization to pool A per unit time. The modified Bloch equations are given by

\[
\frac{dM_A}{dt} = \frac{M_{A0} - M_A}{T_{1A}} - \frac{M_A}{\tau_A} + \frac{M_B}{\tau_B} \tag{2.18}
\]

\[
\frac{dM_B}{dt} = \frac{M_{B0} - M_B}{T_{1B}} - \frac{M_B}{\tau_B} + \frac{M_A}{\tau_A} \tag{2.19}
\]

where \( T_{1A} \) and \( T_{1B} \) are the longitudinal relaxation times as defined above. The general solution to these coupled equations is [48]

\[
M_A(t) = M_{A0} + C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} \tag{2.20}
\]

\[
M_B(t) = M_{B0} + C_1 \frac{R_{1A} + K_A - \lambda_1}{K_B} e^{-\lambda_1 t} + C_2 \frac{R_{1A} + K_A - \lambda_2}{K_B} e^{-\lambda_2 t} \tag{2.21}
\]

where

\[
C_1 = \frac{R_{1A} + K_A - \lambda_2}{\lambda_1 - \lambda_2} (M_A(0) - M_{A0}) - \frac{K_B}{\lambda_1 - \lambda_2} (M_B(0) - M_{B0}) \tag{2.22}
\]

\[
C_2 = \frac{R_{1A} + K_A - \lambda_1}{\lambda_1 - \lambda_2} (M_A(0) - M_{A0}) - \frac{K_B}{\lambda_1 - \lambda_2} (M_B(0) - M_{B0}) \tag{2.23}
\]

and
\[ \lambda_{1,2} = \{(R_{1A} + K_A + R_{1B} + K_B) \pm \]
\[ ((R_{1A} + K_A + R_{1B} + K_B)^2 - 4(R_{1A}R_{1B} + R_{1A}K_A + R_{1B}K_B))^{1/2}\}/2. \] (2.24)

\( R_{1A} \) and \( R_{1B} \) are the reciprocals of the longitudinal relaxation time for pool A and B respectively.

A cross relaxation time, \( T_{cr} \), can be defined between the pools as follows [59]

\[ P_A K_A = P_B K_B = \frac{1}{T_{cr}} \] (2.25)

where \( P_A \) and \( P_B \) are the probabilities of finding a proton in pool A and B respectively.

If the signal from pool B is brought to zero and the \( T_1 \) times for both pools are ignored, then the system will return to equilibrium through a signal increase in pool B from pool A of the form

\[ S(t) = (1 - e^{-t/T_B}). \] (2.26)

Experimentally, \( \tau_B \) is measured and related to \( T_{cr} \).

As an example, white matter is thought to consist of two water pools, myelin water and intra/extracellular water. The amplitudes and \( T_1 \) times have been estimated as 16% and 200 ms for myelin water and 84% and 800 ms for intra/extracellular water. A plot of the change in relative amplitude and \( T_1 \) measured in the presence of cross relaxation as a function of the cross relaxation time is shown in Figure 2.1.

2.4 Magnetization Transfer

Magnetization transfer (MT) is equivalent to cross relaxation and used in MRI [47]. In brain tissue, two pools of protons are assumed, one mobile pool associated with water and one motionally restricted pool associated with macromolecules. There is very little motion involved in the motionally restricted pool so the frequency distribution is broad and the signal decay time is short. In the mobile pool, motion averages the dipole-dipole
Figure 2.1: A white matter model of the change in $T_1$ relaxation time and amplitude of myelin water (labeled with m) and intra/extracellular water (labeled with i) as a function of the cross relaxation time. The true amplitudes and $T_1$ times for the different pools are 16% and 200 ms, and 84% and 800 ms.
interaction and the lineshape narrows with an associated increase in $T_2$ relaxation time. The lineshapes of the two pools are shown in Figure 2.2. These two pools continuously undergo exchange of magnetization.

Figure 2.2: Representation of two proton pools in brain: motionally restricted macromolecules (broad line) and mobile water (narrow line). Both curves are centred on the water resonance, $\nu_0$. Note that the intensity of the mobile pool has been reduced to fit on the plot.

Unlike in $^1$H-NMR, with MRI it is not possible to measure the motionally restricted component of the signal. It is possible however, to perturb the motionally restricted pool and then monitor the effect of magnetization transfer (or cross relaxation) on the mobile signal. If the motionally restricted signal is kept at saturation (signal equals zero), then the $M_B$ term in equation 2.18 disappears and the magnetization in pool A will relax with
Chapter 2. General Theory

a time constant, $T_{1\text{sat}}$, given by

$$\frac{1}{T_{1\text{sat}}} = \frac{1}{T_{1A}} + \frac{1}{\tau_A}. \quad (2.27)$$

This leads to the solution

$$M_A = (M_{Ao} - M_{Asat})e^{-t_{sat}/T_{1\text{sat}}} + M_{Asat} \quad (2.28)$$

where $t_{sat}$ is the time for which pool B is kept saturated and

$$M_{Asat} = \frac{T_{1\text{sat}}}{T_{1A}} M_{Ao}. \quad (2.29)$$

$M_{Asat}$ is the final magnetization attained by pool A if pool B is kept saturated.

There are two methods of saturating pool B. One is the application of a long off-resonance pulse which will only affect the broad macromolecular pool. By continuous application of this pulse, the broad pool continuously undergoes dephasing and therefore, no net magnetization remains. The power spectrum of the sinc pulse is shown in Figure 2.3. The second method is to apply a short on-resonance binomial pulse. The net angular displacement of this pulse is zero so protons with a long $T_2$ will not undergo relaxation and their magnetization will be brought back to its initial position. Protons with short decay times will dephase during the course of the pulse and will not experience a net zero degree pulse but a randomisation of their magnetization. Therefore, the macromolecules, which have short $T_2$s, become dephased while the water is brought back to its initial position. The power spectrum of a binomial pulse superimposed on the spectrum of the two brain proton pools is shown in Figure 2.3.

In practice, it is very hard to attain full saturation of pool B while keeping pool A unaffected. Therefore, compromises are made which cause the above equations to become invalid. Instead a more qualitative approach is taken where the difference in the image intensity before and after the MT pulse is observed and related to the underlying tissue
Figure 2.3: The power spectrum of both a sinc and binomial MT pulse is sketched superimposed on the spectrum of the two proton pools in brain.
structure. This change is quantified by means of a magnetization transfer ratio (MTR) defined as

\[ MTR = \left( 1 - \frac{M_s}{M_0} \right) \times 100\% \]

where \( M_s \) refers to the signal intensity from an image acquired with an MT pulse and \( M_0 \) refers to the signal intensity from an image acquired without an MT pulse. Decreases to the MTR would occur due to a reduction in the macromolecular pool or an increase in the water pool.
3.1 Samples

Two sets of experiments were carried out, one on bovine brain and the other on human brain. The bovine brain was used for \textit{in vitro} $^1$H-NMR experiments. MRI experiments were performed \textit{in vivo} on human brain from normal volunteers and MS patients.

3.1.1 Bovine brain

Bovine brain was obtained within three hours of slaughter (GrandMaison beef farm, 5175-184 St, Surrey) and immediately placed in phosphate buffered saline (Oxoid, lot# R025361-002) cooled with ice. As soon as possible, the brain and buffer were placed in a refrigerator ($4^\circ$C). White and grey matter tissue samples were cut from the brain, dried on a paper towel and placed in a 10 mm o.d. NMR tube. The samples were then placed in an NMR spectrometer and a variety of experiments were carried out. When a sample was not in use, it was stored in the refrigerator. Samples were allowed to warm up to room temperature for half an hour before the experiments were started. Samples were cut from the brain just prior to their placement in the spectrometer. Therefore, samples were removed from the brain over the course of 3-4 days. Three bovine brains were used with 6 samples taken from the first brain (labelled Samples 1-1, 1-2, 1-3, 1-4, 1-5, 1-6), 4 samples from the second brain (labelled 2-1, 2-2, 2-3, 2-4) and 6 samples from the third brain (labelled 3-1, 3-2, 3-3, 3-4, 3-5, 3-6). The composition of the different samples is
Table 3.1: Composition of samples obtained from three separate bovine brains. MG = mostly grey, W = white, G = grey.

shown in Table 3.1. Dehydration experiments were carried out on samples from the third brain by placing the samples in an oven with a drying agent (anhydrous CaSO₄) at 30°C. For complete dehydration, the samples were left under vacuum for 48 hours. Samples were weighed before and after each dehydration step. Moisture contents were calculated as the weight of liquid in the sample over the total weight of the sample. The moisture contents used are shown in Table 3.1.

3.1.2 Human brain

For one experiment (chapter 4), ten normal volunteers (age 20-47) and nine clinically definite MS patients (age 30-56) were examined. Five MS patients were relapsing-remitting (R/R) and four were secondary progressive (SP) in their clinical course. For the second experiment (chapter 5), only normal volunteers were used. More details appear in
3.2 NMR and MRI Equipment

The NMR experiments were performed on a modified Bruker SXP 4-100 NMR spectrometer operating at 90 MHz with a 11μs receiver deadtime. The data acquisition and analysis system included a locally built pulse programmer [60], a Rapid Systems digitizer and an IBM compatible computer. The 90° pulse length was 1.8 μs and the 180° pulse length was 3.9 μs. Temperature was set using a Bruker B-ST 100/700 temperature controller accurate to ±0.5°C.

MRI experiments were done using a 1.5 T General Electric Signa clinical scanner operating at the 5.4 software level.

3.3 NMR Pulse Sequences and Analyses

In this section, all pulse sequences used on the NMR spectrometer are described. The method of analysis associated with each sequence is also mentioned. More details of the individual experiments are described in later chapters.

3.3.1 Free Induction Decay

The free induction decay (FID) was used to separate the signal from solid and mobile protons. The solid signal arose from dipolar coupled protons in macromolecules. The mobile signal came from protons in water or other isotropically moving protons. The pulse sequence was:

\[ 90_x - \tau/2 - (180_y - \tau)s - TR \]
with $\tau = 200\mu s$. The FID signal for dipolar coupled protons can be fit to a moment expansion equation given as:

$$S(t) \approx S(0)(1 - M_2 t^2/2! + M_4 t^4/4! - M_6 t^6/6!)$$  \hspace{1cm} (3.1)$$

where $M_2$, $M_4$, $M_6$ are the second, fourth and sixth moments of the lineshape and $S(0)$ is the total signal at $t=0$. The brain FID decay curve from 17 to 42$\mu$s from the centre of the 90° pulse was fit to this expression using a non-linear function optimisation program minimising $\chi^2$ [61]. The eight 180° pulses were included in order to minimise the effect of magnetic field inhomogeneity. The intensities from the tops of the 8 echoes were fit to a sum of two exponentials and extrapolated to $t=0$ in order to obtain the initial mobile signal, $M(0)$. In order to determine the second moment of only the solid protons, $M'_2$, the mobile signal (assumed to have an $M_2$ of zero) was removed using $M'_2 = M_2 \frac{S(0)}{S(0) - M(0)}$.

The difference in total signal intensity of the FID between two different hydration levels and the corresponding mass difference was used to estimate the proton density of the solid (non-aqueous) brain tissue.

### 3.3.2 Spin-Spin Relaxation

The mobile signal was further characterised using a Carr-Purcell-Meiboom-Gill (CPMG) [62, 63] sequence with 4320 180° pulses and echo spacings of 100 $\mu$s, 200 $\mu$s or 400 $\mu$s. 736 echoes were collected; the first 224 echoes were collected at every echo and the last 512 echoes were collected every 8th echo. The first 4 decay points were discarded since there was a large amount of scatter in the intensity of the initial points [64].

### 3.3.3 Spin-Lattice Relaxation

The spin-lattice relaxation time ($T_1$) was determined using a modified inversion recovery pulse sequence (IR) and a partial saturation recovery pulse sequence (SAT). The IR pulse
sequence was:

\[ 90_x - TR \]

\[ 180_x - \tau - 90_x - TR \]

Eighteen \( \tau \) values were used for IR in brain 1 and 2: 1, 3, 5, 10, 15, 25, 40, 60, 90, 140, 200, 300, 500, 750, 1000, 1500, 2000, 3000 ms. In brain 3, 30 \( \tau \) values were used: 1, 2, 3, 5, 7.5, 10, 15, 20, 25, 30, 40, 50, 75, 100, 125, 150, 200, 250, 300, 400, 500, 750, 1000, 1500, 2000, 2500, 3000, 4000, 5000 ms. The second FID signal was subtracted from the first in order to obtain a resultant positive signal, \( S(t) \), that decayed to zero at long \( \tau \). For a system with only mono-exponential relaxation, this signal decays as \( S(t) = 2M_0e^{-\tau/T_1} \) where \( M_0 \) is the total magnetization along the z-axis. The solid and mobile (assumed to be liquid) parts of the signal were separated for each \( \tau \). The mobile signal was taken as the average signal from 80 to 90 \( \mu s \) after the 90° pulse. The solid signal was found by subtracting the liquid signal from the total signal (averaged between 15 and 25 \( \mu s \) from the 90° pulse).

For the SAT pulse sequence, an FID was used with \( \tau = 200\mu s \) and TRs of: 200, 300, 500, 600, 750, 1000, 1500, 2000, 3000, 4000, 5000, 7000 ms. SAT was only measured for brains 1 and 2. The signal intensity was then fit to the expression \( S(t) = M_0(1-e^{-TR/T_1}) \). This equation assumes a mono-exponential \( T_1 \).

### 3.3.4 Cross Relaxation

The cross relaxation time, \( T_{cr} \), between the solid and mobile protons was measured using the pulse sequence [65]:

\[ 90_x - t_1 - 90_{-x} - \tau - 90_x - TR \]

\[ 90_x - t_1 - 90_x - \tau - 90_x - TR \]
The $t_1$ value was 400$\mu$s which allowed for complete dephasing of the solid signal. The $\tau$ time allowed exchange between the solid and mobile protons causing the reappearance of the solid signal after the third 90° pulse. The $\tau$ times used were 1, 2, 5, 10, 15, 25, 50, 75, 100, 150, 200 ms for brains 1 and 2 and 1, 2, 3, 5, 7.5, 10, 15, 20, 30, 50, 75, 100, 150, 200 ms for brain 3. The first trace was subtracted from the second in order to eliminate $T_1$ effects from the solid signal but not the water. The average signal from points 80 to 90 $\mu$s was subtracted from the average signal from points 16 to 26 $\mu$s in order to characterise the solid signal intensity.

3.3.5 $T_1$-$T_2$ Relaxation Dependence

The two-dimensional $T_1$-$T_2$ dependence was determined with the modified CPMG sequence:

$$90_x - \frac{TE}{2} - (180_y - TE)_n - TR$$

$$180_x - \tau - 90_x - \frac{TE}{2} - (180_y - TE)_n - TR$$

with $TE=200\mu$s and the first 15 $\tau$ values (Brain 1 and 2) or all 18 $\tau$ values (Brain 3) of the Brain 1,2 IR sequence above. The second curve was subtracted from the first curve in order to obtain a positive $T_1$ relaxation signal which decays to zero. The CPMG part of the sequence was collected exactly the same as before (Section 3.3.2). The array of $T_1$-$T_2$ decay data was simultaneously fit using a modified NNLS algorithm [66] with a logarithmically spaced set of 20 $T_1$ times from 0.01 to 5s and 20 $T_2$ times from 0.001 to 2s. This sequence was only performed on white matter samples.
3.3.6 Cross-\(T_2\) Relaxation Dependence

The two-dimensional cross-\(T_2\) dependence was determined with the modified CPMG sequence:

\[
90_x - t_1 - 90_{-x} - \tau - 90_x - \frac{TE}{2} - (180_y - TE)_n - TR
\]

\[
.90_x - t_1 - 90_{-x} - \tau - 90_x - \frac{TE}{2} - (180_y - TE)_n - TR
\]

with \(t_1=400\mu s\), \(TE=200\mu s\) and the same \(\tau\) values as the cross relaxation experiment for Brain 3. The second curve was subtracted from the first curve in order to eliminate \(T_1\) effects from the solid. The CPMG part of the pulse sequence was collected exactly the same as before (Section 3.3.2). Each CPMG curve was analysed in the same manner as the spin-spin relaxation data using NNLS (described in 3.5). The \(T_2\) distributions were found to have 2 or three components assigned to myelin water (\(T_2\) between 1 ms and 30 ms), intra/extracellular water (\(T_2\) between 30 ms and 200 ms) and buffer (\(T_2\) greater than 200 ms). For each \(\tau\) value, the area under the myelin water peak and the area under the intra/extracellular water peak were calculated. This resulted in decay curves of \(\tau\) times and peak areas. These curves were fit to mono-exponential relaxation models. This experiment was applied only to the Brain 3 samples. \(T_1\)-\(T_2\) relaxation curves were also decomposed into 2 or 3 \(T_2\) components in the same manner for comparison to the cross-\(T_2\) decay curves.

3.4 MRI Pulse Sequences and Analyses

MRI experiments included magnetization transfer (MT), 32 echo \(T_2\) relaxation and 32 echo \(T_2\) relaxation with an MT pulse. Each sequence as well as the analysis is described below. In all cases, a transverse slice through the base of the genu and splenium of the corpus callosum was examined. Volumes of interest were outlined in eleven structures,
including five white matter or normal appearing white matter (NAWM) structures: the genu and splenium of the corpus callosum, the posterior internal capsules and the major and minor forceps, and six grey matter structures: the putamen, the head of the caudate nucleus, the thalamus, the cingulate gyrus, the insular cortex and cortical grey matter. Outlines of these regions are shown on an image in Figure 3.1.

Figure 3.1: Image with each brain structure outlined to show ROIs chosen for MRI analysis. On this image, white matter regions are darker on the image than grey matter. A water tube is shown on the right-hand side of the image.
3.4.1 Magnetization Transfer

For the magnetization transfer measurements, a spin echo sequence was used with sequence parameters: repetition time TR 1 s, echo time TE 16 ms, 2 averages, slice thickness 5 mm, FOV 220 mm and matrix size 256x128. Ten slices were acquired but only the slice matching that of the T2 relaxation measurement was used in this analysis. This sequence was run twice, once with no MT pulses and once with two 19 ms sinc pulses at 2 kHz off-resonance with total flip angle amplitude equivalent to a 1060° pulse applied preceding each spin echo. This sequence was optimised to obtain the greatest difference in MTR between normal white and grey matter. MTR images were produced by plotting the MTR at each voxel. A binomial pulse was used in Chapter 5. This pulse consisted of six 1 ms long 121 on-resonance pulses with spacings of 400 μs.

3.4.2 T2 Relaxation

For the T2 relaxation measurements, a single slice 32 echo sequence was used. This sequence consisted of a 90° slice selective pulse followed by 32 rectangular composite 180° pulses flanked by slice-select crusher gradient pulses of alternating sign with descending amplitude for elimination of stimulated echoes and signal from outside the selected slice [67]. Sequence parameters were: repetition time TR 3s, echo spacing 10ms, slice thickness 5mm, FOV 220mm, bandwidth ±32kHz, matrix size 256x128 and 4 averages.

3.4.3 T2 Relaxation with MT

The pulse sequence consisted of the preparatory MT pulse followed by the 32 echo sequence mentioned above (Section 3.4.2). The only difference was that only 2 averages were done. Two types of preparatory MT pulses were employed: 1) two 19 ms sinc pulses at 2 kHz off-resonance (unless otherwise specified) or 2) 6 121 binomial pulses,
each binomial pulse with a duration of 1 ms. Five different studies were carried out and described in chapter 5.

### 3.5 Non-negative Least Squares (NNLS) Analysis of Relaxation

The CPMG (NMR) and 32 echo (MRI) decay curves were decomposed into an arbitrary number of exponentials using a modified non-negative least-squares fitting routine (NNLS) [66]. The range of $T_2$ times included 100 points from 1 ms to 5 s (15 ms to 2 s for 32 echo). Non-negative least-squares analysis gives a discrete $T_2$ distribution made up of delta functions as the lowest $\chi^2$ fit to a decay curve. The true $T_2$ distribution in brain is more likely composed of a continuous distribution of relaxation times. To accommodate this, the $\chi^2$ value was allowed to increase from 1-2% (0.1-0.3% for 32 echo) by minimising solution roughness as well as $\chi^2$ [17, 66, 68]. This produced smooth $T_2$ distributions which were more robust in the presence of noise. A typical $T_2$ distribution is shown in Figure 3.2. The corresponding decay curve is shown in the inset. Both the discrete and smooth $T_2$ distributions are shown. Myelin water percentages were calculated by dividing the intensity of the $T_2$ distribution from 1-50 ms (15-40 ms for 32 echo) by the intensity between 1-700 ms (excluding CSF). Myelin water maps were obtained by calculating the myelin water percentage at each pixel in the image and plotting the intensity. Geometric mean $T_2$ times [17] (mean on a logarithmic scale) for the entire $T_2$ distribution and for the range from 50 to 900 ms (excluding the short $T_2$ component and cerebrospinal fluid) were calculated for MS lesions.

Analysis of the $T_2$ relaxation with MT data was slightly different. Within each white and grey matter structure, regions of interest were drawn and the decay curves from all pixels averaged. The average curve was then decomposed into a maximum of four exponentials at 20, 80, 120 and 2000 ms. The four exponentials were chosen to match
Figure 3.2: Typical $T_2$ distribution for bovine white matter. The corresponding decay curve is shown in the inset. Both discrete (spikes) and smooth (curves) distributions are shown.
the T₂ relaxation values found in our previous brain studies [17, 23]. In these previous studies, decay curves were decomposed into an arbitrary number of exponentials using a modified non-negative least-squares (NNLS) technique. Unfortunately, the lower signal to noise ratio obtained with only two averages for our 32 echo images caused fluctuations in the intensity and position of the short T₂ component. Therefore, the number of degrees of freedom was decreased in order to make the short T₂ component signal more stable. Results from our modified NNLS solution with χ² misfits of 5% greater than the minimum and a myelin window of 15–40 ms gave similar results as the four exponential solutions. Also, replacing the 120 ms T₂ with a 200 ms T₂ did not change the results.

The four proton water pools were separated into the short T₂ component (signal at 20 ms) and the long T₂ component (combined signal from 80 and 120 ms). The total signal was the sum of the short and long T₂ components and signal from CSF (signal with T₂ at 2 s) was ignored. Magnetization transfer ratios were calculated for the short, long and the total T₂ component signals

\[
MTR = \left(1 - \frac{\text{Area}_s}{\text{Area}_o}\right) \times 100\%
\]

where \(\text{Area}_s\) and \(\text{Area}_o\) are the intensities of the given T₂ component or components acquired with and without an MT pulse, respectively.

NNLS was also used to determine T₁ distributions from T₁ decay curves. The range of T₁ times included 100 points from 1 ms to 10 s. The χ² value was allowed to increase from 1-2% to obtain smooth distributions.
4.1 Summary

Magnetization transfer and $T_2$ relaxation experiments were performed on five white and six grey matter brain structures from ten normal volunteers and nine multiple sclerosis patients. Thirty MS lesions were also analysed. Magnetization transfer ratios and myelin water percentages were compared. Both techniques showed a significant difference between the average of white and grey matter of the normal volunteers as well as the average of normal appearing white matter and grey matter of the multiple sclerosis patients. The average magnetization transfer ratio and myelin water percentage for lesions were significantly lower than those of normal appearing white matter in MS patients. Myelin water percentages and magnetization transfer ratios were uncorrelated in white and grey matter but showed a small ($R=0.5, P=0.005$) but significant correlation in multiple sclerosis lesions. In summary, the myelin water percentage and the magnetization transfer ratio provide quantifiable but largely independent measures of multiple sclerosis lesion pathology.

4.2 Introduction

In multiple sclerosis (MS), lesions show up as clearly defined bright areas on proton density or $T_2$-weighted MR images [51, 54, 56]. Unfortunately, it is not possible to determine lesion pathology (e.g. edema, demyelination or gliosis) from conventional MR
images [53]. Two recently developed in vivo MR techniques have the potential to provide more specific information about MS pathology: magnetization transfer (MT) [18] and measurement of the $T_2$ decay curve [16]. Both techniques provide results which vary over a wide range for different lesions and each has been hypothesised to be related to myelination [16, 18, 49, 50, 52, 55].

An early study by Dousset et al. [18] found, in guinea pigs with experimental allergic encephalomyelitis (EAE), that EAE lesions had slightly reduced MT effects and showed no signs of demyelination. They also found, in MS patients, that MS lesions had a very pronounced reduction in magnetization transfer compared to normal white matter. A study showing a more convincing link between MT and demyelination was again done by Dousset et al. [19] who showed histological correlations between MTRs and heavily demyelinated and necrotic lesions in primates injected with lysolecithin. The myelin water percentage has also been found to vary for different multiple sclerosis lesions [16]. Stewart et al. [20] showed that, in guinea pigs with EAE lesions, spinal cord samples with histological evidence of demyelination also showed reduction in the amplitude of the short $T_2$ component.

Both MT and $T_2$ decay curve analyses promise to provide specific information about multiple sclerosis pathology. If the magnetization transfer and the myelin water percentage are both related to myelination, then they should also be related to each other. The goal of this study was to apply both techniques to MS patients and normal volunteers to determine whether the information from these two techniques was correlated.

4.3 Materials and Methods

All experiments were done on a 1.5 T General Electric Signa clinical MR scanner. MT and $T_2$ relaxation (described in Section 3.4) were performed on ten normal volunteers
and nine MS patients. ROIs (as outlined in Section 3.4) were drawn on all images. For each ROI, the MTR and myelin water percentage was determined. In the MS patients, 30 lesions were also examined (16 from R/R patients and 14 from SP patients).

All statistical comparisons were done using the Student's t-test. A two-tailed test was used to determine significant differences and a one-tailed test was carried out to determine significance when comparing normal to MS results since the hypothesis was that results from MS should be lower than results from normal brain. For comparisons between the means from the five different white matter structures, a single factor anova test was used. Significance in all cases was indicated by P values less than 0.05. If a difference in the mean was found from the anova test, Duncan's multiple range test was applied to the white matter structures to determine which pairs were significantly different.

4.4 Results

4.4.1 Normal Volunteers

A typical MTR image and myelin map from a normal volunteer are shown in Figure 4.1 (left and centre respectively). The corresponding proton density weighted image is shown in Figure 4.1 (right). In the MTR image and myelin map, white matter regions appear brighter than grey matter regions consistent with the hypotheses that myelin has a larger pool of motionally restricted protons and contains compartmentalised water.

Figure 4.2 shows MTRs and myelin water percentages for each structure averaged over the ten normal volunteers (closed circles). The average MTR of white matter was significantly different than grey matter (P<0.00001). The anova test between the five white matter structures showed that the mean MTRs were not significantly different. The average myelin water percentage of white matter was significantly different than
Figure 4.1: An MTR image (left), a myelin water map (centre) and a proton-density weighted image (right) from a normal volunteer.
grey matter (P<0.00001). The five white matter structures had significantly different mean myelin water percentages (P<0.00001). Results from Duncan's multiple range test on myelin water percentages are shown in Figure 4.3 with structures written in ascending order and ovals joining structures without significant differences.

The ranking of white and grey matter structures according to their MTR or their myelin water percentage was different. There was no correlation between MTRs and myelin water percentages in white matter (R=0.126, P=0.39) or grey matter (R=-0.014, P=0.9).

4.4.2 MS Patients

An MTR image and a myelin map from an MS patient (R/R) are shown in Figure 4.4 (left and centre respectively). The corresponding proton density weighted image, Figure 4.4 (right), is also given where the lesions are clearly seen as bright areas.

Figure 4.2 shows the average MTR and myelin water percentage for each structure excluding lesions (open circles). The ranking of the white matter structures between MS and normal was almost the same for T₂ relaxation (two structures swapped) but not the same for MT (three structures were ranked differently). None of these changes in rank were significant according to the anova and Duncan's multiple range tests. In MS patients, the average MTR of NAWM and the average MTR of grey matter were significantly different (P<0.00001). The mean MTRs between NAWM structures were not significantly different with the anova test. The average myelin water percentage of NAWM was significantly different than grey matter (P<0.00001). The means of the five NAWM myelin water percentages were significantly different (P<0.00001). Results from Duncan's multiple range test on myelin water percentages are shown in Figure 4.3 with structures written in ascending order and ovals joining structures without significant differences.

As with the normal volunteers, the rankings of white and grey matter structures
Figure 4.2: A plot of the average myelin water percentage (top) and the average MTR (bottom) for five white matter and six grey matter structures. Results from both normal volunteers (closed circles) and MS patients (open circles) are shown. Error bars correspond to the standard error.
Figure 4.3: Duncan’s multiple range test for myelin water percentages from normals and MS patients. Ovals join structures with no significant difference.

Figure 4.4: An MTR image (left), a myelin water map (centre) and a proton-density weighted image (right) from an MS patient.
between MT and T\textsubscript{2} relaxation were not consistent. There was no significant correlation between MTRs and myelin water percentages for NAWM (R=0.154,P=0.3) and grey matter (R=0.253,P=0.065).

4.4.3 Normal Volunteers vs MS Patients

The results from normal volunteers and MS patients were compared. For all white matter structures, NAWM had a lower MTR and lower myelin water percentage than normal white matter. The probability of either of these occurring by chance is only 3%. A one-tailed t-test was used to determine whether MTRs or myelin water percentages from MS structures were significantly lower than normal structures. Structures with significant differences are indicated by a star on Figure 4.2 and include the minor forceps for both MTR and myelin water percentage and the splenium and genu of the corpus callosum for MTR.

4.4.4 Lesions

For this study, the most important comparison is that between MTR and myelin water percentages in lesions. Values are listed in Table 4.1 and plotted in Figure 4.5. The average values of normal white matter, NAWM and grey matter are shown for comparison with lesion values. There was a significant correlation between myelin water percentage and MTR but it was surprisingly moderate (R=0.5,P=0.005). The MTR in lesions was slightly more correlated with the geometric mean T\textsubscript{2} of the entire T\textsubscript{2} distribution (R=-0.59,P=0.0005) and with the geometric mean of T\textsubscript{2} between 50-900 ms (R=-0.54,P=0.001) than with the myelin water percentages.
Comparison of MTRs and Myelin Water Percentages

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<th>Structure</th>
<th>Myelin Water (%)</th>
<th>MTR (%)</th>
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<td>normal</td>
<td>MS</td>
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<tr>
<td>R/R MS</td>
<td></td>
<td>3.8(0.9)</td>
</tr>
<tr>
<td>SP MS</td>
<td></td>
<td>5.6(0.6)</td>
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<tr>
<td>Average lesions</td>
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</tr>
<tr>
<td>Average Grey</td>
<td>3.0(0.3)</td>
<td>3.1(0.3)</td>
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</table>

Table 4.1: Comparison of results for myelin water percentages and magnetization transfer ratios from MS lesions. (Average white and grey are included for reference.) Numbers in parentheses are standard errors.

Figure 4.5: A plot of the myelin water percentage versus the MTR for MS lesions. Lesions from patients with relapsing-remitting MS (closed circles) and from patients with chronic-progressive MS (open circles) are differentiated.
Chapter 4. Comparison of MTRs and Myelin Water Percentages

4.5 Discussion

The main goal of this study was to compare MTR values and myelin water percentages in the brain of normal volunteers and MS patients. Both methods claim to be related to myelin content and therefore one might expect the results to be correlated with one another.

4.5.1 MTR Values

Brain MT measurements in the literature [18, 49, 50, 52, 55, 69, 70, 71, 72, 73] have been acquired in a variety of ways so that absolute MTR values vary considerably from study to study. Because gradient echo sequences use much less rf power to produce an image, the incremental effect of an MT pulse is more potent for gradient echo MT sequences than spin echo MT sequences. Consequently, gradient echo MT sequences produce much larger MTR values for normal white matter. However, a recent abstract by Berry et al. [74] showed that MTRs for normal white matter were linearly related to the effective flip angle (FAsat) divided by the repetition time between saturation pulses (Ts at). Our MTR values for normal white matter, which were obtained with a ratio of FAsat/Tsat equal to 10.6, fell onto Berry’s linear relation within experimental error. Therefore, although our spin echo MT sequence yielded smaller white matter MTR values than many other studies, especially those obtained with gradient echo MT sequences, our results should be representative of all MT studies.

4.5.2 Comparison of Our MTRs and Myelin Water Percentages with Other Studies

Several groups have reported MTR values from different normal white matter regions [50, 52, 55, 69, 71, 72]. The ranking of their white matter structures was the same as ours except in one case. Although, Mehta et al. [72], like us, found that the genu of the
corpus callosum had the largest MTR, the other structures were ranked differently. This group was the only one to use an on-resonance binomial MT pulse which may account for the difference in ranking.

Three groups compared normal white matter to grey matter and found a significant difference in the average MTR [18, 50, 72] consistent with our results. In MS patients, three groups found that the average MTR of NAWM was lower than normal white matter [18, 49, 52], as did our group, but one group did not find it significant [50]. Several groups examined MS lesions and all found that the average MTR of lesions was significantly lower than NAWM [18, 49, 50, 70], as was found in our experiments. There was a wide range of MTR values for the lesions. These lesions were further separated into lesions from patients with relapsing-remitting and secondary progressive MS. Three of the groups did not find a significant difference in the MTR between the two MS classes [49, 50, 70] but one group found that lesions from R/R patients had a higher MTR than lesions from SP patients [18]. This result is the opposite of ours; we found that lesions from SP patients had a higher MTR than lesions from patients with R/R MS. This finding could be due to the large variation in types and ages of lesions found within a single MS patient. In SP MS, lesions are expected to be older than lesions from R/R patients. Tomiak et al. [73] found that lesions older than one year had a larger MTR than younger lesions. They attributed this result to older lesions being mainly gliotic and therefore having less water to exchange with the motionally restricted protons. The findings in that study would support a larger MTR in lesions from SP patients as opposed to R/R patients.

Only one other systematic study of myelin water percentages has been carried out with normal volunteers. In our earlier study [17], the average myelin water percentage was 11.3% in white matter and 3.1% in grey matter for normal volunteers. These values were not significantly different than our present results even though the previous study was carried out on a different scanner and employed a different slice thickness (10 mm).
The ranking of the white matter structures was the same as this study except for the major forceps. In the previous study, the optic radiation was included in the major forceps but, in this study, since the slice chosen for most MS patients did not include the optic radiation, it was not included. Grey matter structures were ranked slightly differently probably due to the small myelin water percentage in grey matter being more affected by the noise.

4.5.3 Other Comparisons of MT and T\(_2\) Results

In a recent study of maturing brains [75], a qualitative inverse correlation was found between \(T_2\) and MTR for brains at different myelination stages. The mono-exponential \(T_2\) measured in that experiment was estimated from a dual echo pulse sequence and therefore cannot be directly related to the \(T_2\) times or myelin water percentages reported in this study.

A comparison between \(T_2\) times measured from a 16 echo sequence and MTRs in lesions from patients with optic neuritis showed a significant correlation (\(r=-0.413, P<0.05\)) [40] which is similar to our results.

4.6 Concluding Remarks

In a clinical setting, the MT sequence has advantages over \(T_2\) measurements since it is easy to implement in a multi-slice fashion and has shorter acquisition times. The \(T_2\) relaxation was measured on a single slice because a multi-slice study would have corrupted the \(T_2\) decay curves with MT effects due to off-resonance irradiation from other excited slices.
T₂ relaxation results show two distinct water compartments within the mobile component of normal white matter. This finding suggests that the two-pool model normally employed for characterising MT is inadequate; a three pool model (macromolecules, myelin water and extracellular/intracellular water) would be more representative. Magnetization transfer is expected to be stronger for myelin water than the intracellular/extracellular water. Furthermore, direct saturation effects, which become more prominent as the MT offset frequency is moved closer to resonance, should be different for the two mobile pools since they have different linewidths.

Further studies are required to determine how MT and T₂ relaxation results relate to brain pathology. If both techniques are indeed primarily measuring myelin content, then we would expect the MTRs and myelin water percentages to be strongly correlated. This study demonstrates that the relationship is much more complex since, in MS lesions, only about one fifth of the variance in the myelin water percentage was accounted for by the MTR. We found a slightly higher correlation between MTR and geometric mean T₂ than with the myelin water percentage. If the myelin water percentage is indeed a measure of myelin content, then the MTR must also be dependent on other aspects of the tissue.

In summary, the MTR and myelin water percentage provide quantifiable but largely independent measures of MS lesion pathology. More investigation is required before we can reliably relate these two parameters to the actual pathological changes.
Chapter 5

MT Effects on the Short and Long $T_2$ Relaxation Components of Brain

5.1 Summary

A $T_2$ relaxation sequence was modified by including a preparatory magnetization transfer pulse in order to determine the MT effect on each of the two $T_2$ components separately. Two types of MT pulses were examined: an on-resonance binomial pulse and an off-resonance sinc pulse. The effect of varying the offset frequency of the MT sinc pulse was also determined. The time between the sinc MT prepulse and the initiation of the $T_2$ relaxation sequence was increased and the effect on each $T_2$ component was seen. The MT effect was significantly larger on the short $T_2$ component than the long $T_2$ component ($P<0.00001$ to $P<0.04$). This differential MT effect was removed at time delays of greater than 500 ms. The binomial MT pulse and small frequency offsets caused substantial direct saturation which preferentially affected the short $T_2$ component. In summary, MT affects the short $T_2$ component in brain more than the long $T_2$ component except at long delays between the MT pulse and the initiation of the $T_2$ relaxation sequence.

5.2 Introduction

Most models of MT in the brain have included only two pools of protons [47, 72, 76, 77, 78, 79], motionally restricted and mobile. The “bulk” water (mobile) interacts with the macromolecular matrix (motionally restricted) through some water surface layer but all the water has equal access to the matrix. From $T_2$ relaxation, it is clear that the
mobile pool can be further separated into two distinct water pools. Therefore, a model including at least 3 proton pools should be used to better understand the MT signal [80]. Exchange would take place between the two water pools and between the myelin water and the motionally restricted pool (consisting mainly of myelin).

In normal MT sequences, the signal from the two tissue water pools is not separable and therefore some sort of average MT is measured. However, a 32 echo sequence preceded by an MT pulse is able to distinguish the MT effect on each of the $T_2$ components.

The goal of this study was to look for differences in magnetization transfer effects between the two brain tissue water pools. Since the microscopic local environment for water between myelin bilayers is very different from that of intra and extracellular water, one might expect the two pools to exhibit different MT effects. The experimental protocol consisted of a preparatory MT pulse applied before a 32 echo $T_2$ relaxation measurement sequence. Two types of MT pulses were investigated: namely an on-resonance binomial pulse and a 19 ms off-resonance sinc pulse. With the sinc pulse, the effect of changing the offset frequency of the MT pulse was investigated. Also, the effect of varying the delay between the end of the sinc MT pulse and initiation of the multiecho $T_2$ measurement sequence was examined. MnCl$_2$ doped water tubes with $T_2$s of 20 and 80 ms were also placed within the image. The choice of water $T_2$ was in order to mimic the previously measured $T_2$ of the different water pools in the brain [16, 17] namely myelin water and intra/extracellular water.

5.3 Material and Methods

MRI measurements were done on a 1.5 T General Electric Signa clinical MR scanner operating at the 5.4 software level. Four MnCl$_2$ doped water tubes (two with $T_2$ 80 ms and two with $T_2$ 20 ms) were placed next to the head of each volunteer.
Relaxation decay curves for all experiments were fit to a 4 $T_2$ model using an NNLS algorithm as detailed in Section 3.4. The relaxation times were then separated into the short $T_2$ component ($T_2 = 20 \text{ ms}$) and the long $T_2$ component ($T_2 = 80 + 120 \text{ ms}$). In the case of the reproducibility data, decay curves were also fit using the modified NNLS algorithm with a 0.1-0.3\% increase in $\chi^2$.

All statistical comparisons were done using the two-tailed Student's t-test. Probabilities less than 0.05 were considered significant. If a structure had no short $T_2$ component when no MT pulse was present, then the person was not included in any average for that structure or the t-test calculation. Also, if an MTR resulted in a negative value (likely due to noise), the MTR was set to zero and included in the average. This occurred in one white matter structure for two different volunteers using the sinc MT pulse. In the experiment with a varied frequency offset, this occurred in the minor forceps for one volunteer (at 500, 750 and 1000 Hz) and in the genu (at 750 Hz). In the experiment where a delay was put between the MT pulse and the 32 echo sequence, negative MTRs were found in the splenium and minor forceps for delays $\geq 500 \text{ ms}$. These negative MTR values ranged from -1.2 to -55 with an average of -12.6.

Five different experiments using the $T_2$ relaxation with MT sequence were done and described below.

### 5.3.1 Reproducibility

Seven experiments with a sinc MT pulse were repeated at the same sitting on one volunteer as well as one case with no MT. Each white matter structure was analysed using the normal 0.1-0.3\% smoothing NNLS algorithm and the 4 $T_2$ method described in chapter 3. This was used to test whether the 4 $T_2$ method was appropriate for relaxation analysis.
5.3.2 Binomial MT pulse

Fifteen normal volunteers (age 25–48) were studied using a binomial MT pulse followed by a 32 echo train. Experiments were done in pairs, without and with MT. MTRs were then determined for both the short and long $T_2$ components. Only one volunteer was scanned with the four water tubes.

5.3.3 Sinc MT pulse

Eleven normal volunteers (age 23–48) were studied using a sinc MT pulse followed by a 32 echo train. The delay between the end of the sinc MT pulse and the initiation of the 32 echo train was 18.5 ms and the frequency offset was 2000 Hz. Experiments were done in pairs, without and with MT. MTRs were then determined for both the short and long $T_2$ components.

5.3.4 MT Frequency Offset

A total of three scans were done on two normal volunteers (i.e. one volunteer was done twice) using a range of off-resonance offsets for the sinc MT pulse. Offsets used were 2000, 1000, 750, 500 and 300 Hz. Only one of the scans included the 300 Hz experiment while only the other two scans included the 750 Hz experiment. Again, an experiment with no MT was done in order to allow the calculation of MTRs at each offset.

5.3.5 Delay Between MT Pulse and 32 Echo Sequence

One normal volunteer was studied twice using a protocol that varied the time between the end of the sinc MT pulse and the initiation of the 32 echo sequence, called the MT delay. Experiments consisted of a sequence with no MT and five other sequences with delays of 18.5, 218.5, 518.5, 1018.5 and 2018.5 ms. MTRs were determined for each delay.
Table 5.1: Amplitudes of the short and long $T_2$ components for the smooth and $4\ T_2$ model distributions in normal white matter. Standard errors are given in parentheses.

5.4 Results

5.4.1 Reproducibility

Seven experiments were repeated on one volunteer in order to determine the reproducibility. The amplitudes of the short and long $T_2$ components are shown in Figure 5.1 and Table 5.1 comparing the normal 0.1-0.3\% smoothing NNLS solution and the $4\ T_2$ method. In all cases except for the splenium, the $4\ T_2$ analysis resulted in a smaller amplitude range. In the case of the splenium, movement between scans is thought to have affected the results thereby leading to unreliable comparisons. The amplitude resulting from the $4\ T_2$ model was always near the middle of the range of amplitudes for the smooth analysis. All other experiments were analysed using the $4\ T_2$ model.

A typical example of both $T_2$ distributions determined with and without MT is shown in Figure 5.2. Two cases are shown: one using a smooth NNLS solution (smooth curves) and the other using the $4\ T_2$ fit to the same data (spikes). The solid lines (labelled no MT) refer to the 32 echo experiment without an MT pulse present and the dotted lines (labelled MT) refer to the 32 echo with MT experiment. The MT axis has been shifted in order to make them visible beneath the no MT lines. A decrease in intensity can be seen between the no MT and MT distributions.
Figure 5.1: The difference between the smooth and 4 T$_2$ model is shown for one no MT (solid symbol) and seven MT (open symbol) cases from white matter of one volunteer. Data in the lower plot comes from the short T$_2$ component and data from the upper plot comes from the long T$_2$ component. Note the change in scale along the vertical axis between the two plots.
Figure 5.2: Example of $T_2$ distributions without and with an MT pulse. The curved solutions were obtained by fitting the decay curves using the modified smooth NNLS algorithm. The spiked solutions were obtained by restricting the allowed $T_2$ values to 4. The no MT curves refer to 32 echo experiments without an MT pulse and the MT curves are experiments with an MT pulse. Note the axis of the MT 4 $T_2$ solution was shifted slightly to the right to make it visible under the no MT solution.
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<table>
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<td>Minor Forceps</td>
<td>Binomial</td>
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<tr>
<td></td>
<td>Minor Forceps</td>
<td>22.4(3.6)</td>
<td>7.4(0.8)</td>
<td>10.4(0.6)</td>
<td>0.007</td>
</tr>
<tr>
<td>Sinc</td>
<td>Major Forceps</td>
<td>23.6(5.4)</td>
<td>7.9(0.7)</td>
<td>9.9(0.6)</td>
<td>0.032</td>
</tr>
<tr>
<td></td>
<td>Internal Capsules</td>
<td>23.6(4.0)</td>
<td>6.6(0.9)</td>
<td>10.0(0.6)</td>
<td>0.005</td>
</tr>
<tr>
<td>Average White</td>
<td>23.1(2.9)</td>
<td>7.8(0.8)</td>
<td>10.6(0.6)</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>80 ms tube</td>
<td>-</td>
<td>-</td>
<td>2.4(0.8)</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>20 ms tube</td>
<td>-</td>
<td>-</td>
<td>4.8(0.7)</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2: Average MTRs of white matter structures for 15 volunteers using binomial (top) and sinc (bottom) MT pulses (offset=2000 Hz and delay=18.5 ms). Decay curves were analysed using the 4 T\textsubscript{2} model. P values refer to a comparison between short and long T\textsubscript{2} components. Numbers in parentheses are standard errors.

5.4.2 Binomial MT pulse

Fifteen normal volunteers were examined using the binomial MT pulse sequence. The average MTR for the short and long T\textsubscript{2} components and the average total MTR is shown in the top half of Table 5.2 for each structure. P values comparing the MTRs from short and long T\textsubscript{2} components are also given. The MTR of the short T\textsubscript{2} component was significantly higher than the MTR of the long T\textsubscript{2} component for all white matter structures. The signal from the 20 ms and 80 ms water tubes was lower due to the binomial pulse with the 20 ms water tube showing a larger MTR.
5.4.3 Sinc MT pulse

In this experiment, eleven normal volunteers were scanned with the sinc MT pulse sequence. The average MTR for the short and long \( T_2 \) components and the average total MTR is shown in the bottom half of Table 5.2 for each structure. P values comparing the MTRs from short and long \( T_2 \) components are also given. The MTR of the short \( T_2 \) component was significantly higher than the MTR of the long \( T_2 \) component for all white matter structures. The average MTR of the 20 ms water tube was significantly higher than that of the 80 ms water tube although the effect was small in both.

5.4.4 MT Frequency Offset

The effect on each of the \( T_2 \) components of varying the offset frequency of the MT pulse was determined. Results for both short and long \( T_2 \) components are shown in Table 5.3 for each white matter structure and in Figure 5.3 for the average over the five structures. At all offset frequencies, the MTR for the short \( T_2 \) component (excluding the minor forceps at 750 Hz) was larger than that for the long \( T_2 \) component. As the offset frequency was decreased to 300 Hz, the absolute increase in MTR for the short \( T_2 \) component became much larger than that for the longer component but the fractional increase was about the same. The reason for the dip in MTR at 750 Hz is not understood. The increase in calculated MTR with decreasing offset frequency was much larger for the 20 ms \( T_2 \) water tube than for the 80 ms water tube.

5.4.5 Delay Between MT Pulse and 32 Echo Sequence

The length of the delay between the sinc MT pulse and the initiation of the 32 echo sequence was varied from 18 ms to 2 s in five steps. Results for both the short and long \( T_2 \) components for each white matter structure are shown in Table 5.4. For both \( T_2 \)
Table 5.3: Average MTRs of white and grey matter structures using different off-resonance offsets. Short and long component water tube MTRs are for the 20 ms and 80 ms tubes respectively.
Figure 5.3: A plot of the average MTR of the short and long $T_2$ components in white matter as a function of off-resonance offset. The data were collected from three scans of two volunteers.
Table 5.4: Average MTRs for the short and long $T_2$ component in brain using different MT delays. Short and long components water tube MTRs are for the 20 ms and 80 ms tubes respectively.

components, the MTR at short delays was larger than that at longer delays. For delays of 200 ms or less, the MTR of the short $T_2$ component was much larger than the MTR of the long $T_2$ component. For delays of 500 ms or greater, the MTR values of both $T_2$ components were similar. Figure 5.4 shows the average MTR for both $T_2$ components in white matter as a function of the delay. Neither water tube showed appreciable change in MTR although the 80 ms water tube, for unknown reasons, had an increased MTR at longer delay times.
Figure 5.4: A plot of the average MTR of the short and long $T_2$ component in white matter as a function of MT delay. The data were collected from two scans of the same normal volunteer.
5.5 Discussion

The conventional method of analysing $T_2$ relaxation data was not employed because the amplitudes for the two $T_2$ components were not stable probably due to a low signal to noise ratio. Instead, a model which incorporated only 4 $T_2$ times at 20, 80, 120 and 2000 ms was fit to the decay curves. These values were chosen to correspond to $T_2$ values measured in normal brain. The amplitudes associated with each $T_2$ component had much less variance than the smooth model and the values were centred within the amplitude range of the smooth solutions. It is important to note that although this model is helpful in normal human brain, disease can cause changes in relaxation time and this model would no longer be valid.

Since it is impossible to separate the contributions to the MTR from each of the two tissue water pools in a conventional MT sequence, the measured MTR is a weighted average over the short and long $T_2$ proton signal components. By combining the preparation MT pulse with a 32 echo sequence, this study separately measured the MTR for each water pool. The MTR of the short $T_2$ component was found to be significantly larger ($P<0.00001$ to 0.04) than that of the long $T_2$ component for all white matter structures and for both types of MT preparation pulses. A larger MTR is an indication of closer association between water and motionally restricted non-aqueous protons such as lipids and proteins. Therefore, the differential MTR effects observed here support the assignments of the short $T_2$ component to water trapped between myelin bilayers and the longer $T_2$ component to water from intra and extracellular water.

Another possible difference between the two water pools is the amount of direct saturation due to an MT pulse. Direct saturation is a decrease in magnetization of the water pool due to direct absorption from the MT pulse and not as a result of transfer of magnetization between proton pools. In an ideal experiment, there should be no direct
saturation but this is never the case. The two doped water tubes were included in the image slice in order to look for direct saturation effects in the MT experiments. One would expect direct saturation effects in tissue to be similar to those in water phantoms possessing similar T\(_1\) and T\(_2\) times. The 20 ms T\(_2\) tube (T\(_1\) = 800 ms) represented the short T\(_2\) component observed in brain and the 80 ms T\(_2\) (T\(_1\) = 200 ms) tube represented the long T\(_2\) component. We note that when the offset frequency of the MT pulse was decreased, the 20 ms component was affected much more than the 80 ms component. Similar direct saturation effects are expected for the short and long T\(_2\) components \textit{in vivo}.

Comparison between the MTR results from binomial and sinc MT pulses show an interesting difference. With the binomial MT pulse, the MTRs for the short T\(_2\) component varied among the different white matter structures. On the other hand, the MTR values obtained with the sinc MT pulse did not show this variance. The MTR for the long T\(_2\) component showed no significant variation for either type of MT pulse. The ranking of binomial pulse MTRs for the short T\(_2\) component coincide with the ranking of the myelin water percentages in Chapter 4 for different white matter structures. These different behaviours with the two MT pulses is tentatively attributed to direct saturation. The binomial MT pulse produces a very large amount of direct saturation as measured from the water standards (Table 5.2) and this direct saturation preferentially affects the myelin water. This direct saturation effect is accentuated at higher myelin water contents. Since the measured MTR is a combination of the MT effect and direct saturation, different white matter structures will have different MTRs. The sinc MT pulse did not produce much direct saturation and therefore the measured MTR values were simply due to MT which appeared to be uniform among different white matter structures. This similarity between white matter MTRs was also found in Chapter 4 where a sinc pulse was used.
Recently, many studies have employed off resonance MT pulses with the off-resonance MT pulse moved to smaller frequency offsets since this leads to larger MTRs [81, 82]. This could also cause more direct saturation of the mobile pool. This was found experimentally and in particular, water with a short $T_2$ was preferentially saturated at small offset values (water tubes in Table 5.3). Therefore, in brain white matter, the myelin water will be more affected by small offsets than intra/extracellular water. For clinical applications this effect may be desirable, however it should be appreciated that the measured MTR at small offsets will be a combination of MT and the different direct saturations of the water. In the previous chapter, the offset used for measurement of MTRs was 2 kHz and therefore a negligible amount of direct saturation was expected.

Only one study has previously combined a CPMG sequence with an MT pulse and it was carried out on bovine white matter [80]. Although they found two $T_2$ components in white matter, they found no difference in their MT effect. This result seems to conflict with those of the present study, however the experimental approaches of the two studies were different. The MT prepulse used in the bovine brain study was a 7 s long rectangular pulse designed to produce complete saturation of the motionally restricted proton pool. During this 7 s, the two water $T_2$ components would have had ample time to exchange magnetization and thereby average the overall MT effect. Hence, a common average MTR was measured for the two components. In our experiment, the two 19 ms sinc pulses (or the six 1 ms binomial MT pulses) did not accomplish complete saturation of the motionally restricted protons and the 32 echo $T_2$ measurement sequence was applied before the two tissue water pools in white matter had sufficient time to come to equilibrium. This hypothesis is further supported by the MT delay experiment. At shorter delays, MTRs were different for the short and long $T_2$ components. At longer delays, the MTR of each $T_2$ component was essentially the same indicating that mixing of the two pools had averaged out the magnetization.
The MT experiment in white matter involves several exchange processes: a separate exchange of magnetization between motionally restricted protons and water in each of the two tissue water pools, and exchange of magnetization between the two tissue water pools. This work enables us to establish crude limits for these exchange times. Exchange between motionally restricted protons and water is believed to occur rapidly since a magnetization transfer effect can be measured after a delay of about 20 ms from the end of the sinc MT pulse. Exchange between the tissue water pools is much slower. The timescale for exchange between the two water pools must be long on a $T_2$ timescale (i.e. greater than 100 ms) since we observe the separate $T_2$ relaxation components for each pool. However, exchange must be fast on a $T_1$ timescale (less than 700 ms) since only one relaxation component is found. Also, the differential MT effect between the short and long $T_2$ components disappears after about 500 ms.

5.6 Concluding Remarks

In all cases (except for long MT delays of 1–2 s), the MTR for the short $T_2$ component was significantly larger than the MTR for the long $T_2$ component. This was expected due to the closer interaction between myelin water and myelin than between non-myelin and intra/extracellular water. The introduction of a delay between the MT and 32 echo pulse sequences allowed the magnetization of the two water pools to equilibrate and only one MTR was found. This occurred by 500 ms of delay showing that the two water pools exchange in a time faster than 500 ms but slower than 100 ms (the $T_2$ of intra/extracellular water). These results show the importance of using a model with more than 2 proton pools when trying to explain MT in brain. These results also demonstrate the difference between using a binomial and a sinc MT pulse to produce saturation. With binomial pulses, white matter structures are found to have different MTRs which
correspond to the same ranking as myelin water percentages. Therefore, binomial pulses, which produce both MT and direct saturation, may be more sensitive to differences in myelin water content. Small frequency offsets for the sinc MT pulse also produce a differential MT effect on the two water pools which is likely due to a combination of MT and direct saturation. Exploitation of this effect may improve contrast between myelin water and intra/extracellular water which in turn may help in visualising pathology such as multiple sclerosis lesions.
Chapter 6

Relaxation Measurements of Bovine Brain using Magnetic Resonance

6.1 Summary

*In vitro* relaxation times were measured from white and grey matter samples excised from three different bovine brains. T<sub>2</sub> relaxation distributions showed 4 peaks which were attributed to buffer solution (T<sub>2</sub> > 500 ms), intra/extracellular water (T<sub>2</sub> between 50 ms and 500 ms) and 2 to myelin water (T<sub>2</sub> < 50 ms). The myelin water percentage was larger for white matter (average=14.3±0.9) than grey matter (average=4.7±0.6). T<sub>1</sub> relaxation of the solid signal was mono-exponential for grey matter but bi-exponential for white matter. A T<sub>1</sub>-T<sub>2</sub> dependent measurement showed that there was no cross relaxation between the different water pools as distinguished by T<sub>1</sub> and T<sub>2</sub>. Cross relaxation times in fully hydrated white matter were measured to be 193 ms between intra/extracellular water and non-myelin molecules and 66 ms between myelin water and myelin. These experiments showed that a 4-pool model with cross relaxation between the water and macromolecules but no exchange between the two water pools (within 1 s) is appropriate when describing bovine white matter. In grey matter, the cross relaxation time was measured to be about 200 ms between the intra/extracellular water pool and the non-myelin molecules.
Chapter 6. Relaxation Measurements of Bovine Brain using Magnetic Resonance

6.2 Introduction

Many biological systems have been examined using $^1$H nuclear magnetic resonance (NMR) and in particular spin-spin ($T_2$) and spin-lattice ($T_1$) relaxation. These techniques allow the differentiation of different proton pools. Previous in vivo $T_2$ relaxation studies [16, 17] have shown that water in human brain has three different relaxation times which are associated with three different water compartments: cerebrospinal fluid ($T_2 >1$s), intracellular and extracellular water ($T_2$ between 80 and 120 ms) and water tightly associated with myelin bilayers ($T_2 < 50$ ms). In vivo measurements on human white matter have shown mono-exponential $T_1$ relaxation [17].

In vitro experiments are advantageous since signal from both the motionally restricted and mobile protons can be separated. Previous studies on guinea pig brain [20] and crayfish abdominal nerve cord [22] showed multicomponent $T_2$ relaxation again associated with different water pools.

This study examined bovine white and grey matter using a wide variety of NMR pulse sequences. Mobile and solid fractions were determined as well as the second moment of the solid signal. $T_1$ and $T_2$ relaxation times were measured and, for white matter, their interdependence. Cross relaxation times between different water and macromolecular pools were found. Finally, NMR properties were studied as a function of tissue hydration. The purpose of this study was to characterise the behaviour of water within the brain, to separate the $^1$H-NMR signal into different solid and mobile (water) components and to determine interactions between the solid and mobile protons as well as the mixing of different water pools.
6.3 Material and Methods

6.3.1 Samples

Samples from three bovine brains were used as indicated in Section 3.1.1. Their composition is shown in Table 3.1

6.3.2 NMR Experiments

A variety of NMR experiments were conducted on the brain samples. These included FID, IR, SAT, cross relaxation, $T_1$-$T_2$ relaxation and cross-$T_2$ relaxation experiments. The repetition time, TR, for all experiments was 7 s unless otherwise stated. Sample 1-2 and 1-5 were measured at 37°C using free induction decay, CPMG and inversion recovery experiments (described in Section 3.3). All other experiments were carried out at 24°C. The integrity of the samples was monitored by repeating the free induction decay and CPMG sequences at least once per day. No changes were seen during the course of the experiments (less than 1 week). Standard errors are presented in parentheses.

6.4 Results

6.4.1 Free Induction Decay

The FID was collected for all samples. Typical FIDs for white and grey matter are shown in Figure 6.1. The top two curves represent fully hydrated samples and the lower two curves represent dehydrated samples. The total mobile and solid signals and the second moment were determined from the moment expansion. The average second moment for white matter was $1.86(0.15) \times 10^9$ s$^{-2}$ and for grey matter $1.99(0.13) \times 10^9$ s$^{-2}$ at an ambient temperature of 24°C. This was slightly larger than the second moments measured from the white and grey samples at 37°C, $1.31 \times 10^9$ s$^{-2}$ and $1.38 \times 10^9$ s$^{-2}$
respectively. The second moment at different moisture contents for both white and grey matter samples is shown in Figure 6.2. From the initial rapid decay of the FID, the solid signal fraction was found to be significantly larger in white matter than grey matter. The average water content of fully hydrated white matter was 72.1(1.1)% and grey matter 84.4(0.9)% as measured from the weight at full hydration and at complete dehydration. From the changes in mass and signal intensity during the hydration study, the average proton density for the solid was found to be 0.0811(0.007) gH/gSolid for white matter and 0.0578(0.018) gH/gSolid for grey matter.

Figure 6.1: Free induction decays for white (solid) and grey (dashed) matter samples. The upper two curves are for fully hydrated samples (71.0% for white and 83.8% for grey) and the lower two curves are for dehydrated samples (0.3% for white and 0.4% for grey). Note that only the first four echoes are included in order to better resolve the initial rapid decay.
Figure 6.2: A plot of second moment ($M'_2$) versus moisture content for white matter (open circles) and grey matter (closed circles).
6.4.2 CPMG

Typical $T_2$ distributions calculated from the $\tau=200\text{ms}$ decay curves for white and grey matter samples are shown in Figure 6.3. The results of fully hydrated samples consistently showed four or five peaks with $T_2$ ranges of 2-8, 10-70, 80-120 and $>120$ ms. $T_2$ distributions at $\tau=200$ and 400 $\mu$s showed similar behaviour whereas at $\tau=100\mu$s, the distribution was much broader (Figure 6.4). The myelin water percentages calculated for each sample are shown in Table 6.1. The myelin water percentages for fully hydrated samples were significantly higher in white matter than grey matter ($P<0.00002$). As the tissue was dehydrated, the $T_2$ of the peaks shifted to lower values and the intensity of all the peaks decreased. Dehydration of white and grey matter samples is shown in Figure 6.5.

![Figure 6.3: Typical $T_2$ distributions for white (solid) and grey (dashed) samples at full hydration.](image-url)
Figure 6.4: T₂ distributions for a hydrated white matter sample at 3 different τ values. The τ = 100 μs curve was only smoothed by 0.1-0.3% whereas the τ = 200 and 400 μs were smoothed by 1-2%.
<table>
<thead>
<tr>
<th>Sample (MC%)</th>
<th>White myelin water (%)</th>
<th>Sample (MC%)</th>
<th>Grey myelin water (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>17.4</td>
<td>1-1</td>
<td>6.4</td>
</tr>
<tr>
<td>1-3</td>
<td>11.1</td>
<td>1-4</td>
<td>5.8</td>
</tr>
<tr>
<td>1-6</td>
<td>15.7</td>
<td>1-5</td>
<td>4.9</td>
</tr>
<tr>
<td>2-1</td>
<td>12.1</td>
<td>2-2</td>
<td>4.6</td>
</tr>
<tr>
<td>2-3</td>
<td>17.5</td>
<td>2-4</td>
<td>6.6</td>
</tr>
<tr>
<td>3-1 (71.0)</td>
<td>15.0</td>
<td>3-3 (83.8)</td>
<td>2.2</td>
</tr>
<tr>
<td>3-2 (73.2)</td>
<td>11.4</td>
<td>3-4 (77.0)</td>
<td>3.7</td>
</tr>
<tr>
<td>3-2 (68.2)</td>
<td>11.5</td>
<td>3-4 (52.8)</td>
<td>4.6</td>
</tr>
<tr>
<td>3-2 (59.9)</td>
<td>10.6</td>
<td>3-5 (85.4)</td>
<td>5.4</td>
</tr>
<tr>
<td>3-2 (37.7)</td>
<td>10.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 (72.0)</td>
<td>14.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 (36.0)</td>
<td>14.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>average</strong></td>
<td><strong>14.3 (0.9)</strong></td>
<td><strong>average</strong></td>
<td><strong>4.7 (0.6)</strong></td>
</tr>
</tbody>
</table>

Table 6.1: Myelin water percentages for white and grey matter samples. Moisture contents are included for brain 3 samples. The average values are for fully hydrated samples only and the standard error is included in parentheses.
Chapter 6. Relaxation Measurements of Bovine Brain using Magnetic Resonance

Figure 6.5: \( T_2 \) distributions at different moisture contents. Plots on the left-hand side are from a white matter sample and plots on the right-hand side are from a grey matter sample. (Note that the amplitude of the fully hydrated sample has been reduced by a factor of 2.)
6.4.3 \( T_1 \) Relaxation

An example of the \( T_1 \) distribution for white and grey matter is shown in Figure 6.6A and B. Both the liquid and solid intensities are included. In grey matter, the solid peak overlapped the liquid peak whereas in white matter, the solid peak was shifted to the left of the liquid peak. The \( T_1 \) distributions as a function of moisture content are shown in Figure 6.7.

Saturation recovery curves for white and grey matter are shown in Figure 6.8. Mono-exponential fits to the data are also shown.

6.4.4 Cross Relaxation

The reappearance of the solid signal as a function of \( T_1 \) for a \( 90 - \tau - 90 - T_1 - 90 \) experiment [65] is shown in Figure 6.9 for a white and grey matter sample. This signal was then fit to the form

\[ S(t) = a + b(1 - e^{-\tau/Ts}) \]

where \( a \) is the correction for the \( T_2 \) decay of the mobile signal at short times and \( b \) is proportional to the amplitude of the solid signal. The measured \( Ts \) is related to the cross relaxation time, \( T_{cr} \), by:

\[ \frac{1}{T_{cr}} = \frac{P_s}{T_s} = \frac{P_w}{T_w} \]

where \( P_s \) and \( P_w \) are the probability of a proton being in the solid and water fraction respectively and \( T_w \) is the water relaxation time due to cross relaxation. A schematic of the different proton pools in bovine brain is shown in Figure 6.10. The signal intensity from each pool was defined as \( S_{mw} \) for myelin water (the area under the \( T_2 \) distribution from 1-50 ms), \( S_m \) for solid myelin (half of the solid FID signal), \( S_{iw} \) for intra/extracellular water (the area under the \( T_2 \) distribution from 50-200 ms) and \( S_{nm} \) for non-myelin macromolecules (half of the solid FID signal). Since the signal intensity, in the absence of relaxation, is directly proportional to the number of protons in a pool, these intensities can be used to determine \( P_s \). In bovine brain, two different types of cross relaxation were
Figure 6.6: Typical $T_1$ distributions showing the separation of liquid and solid protons. Plot A is from a white matter sample and plot B is from a grey matter sample both at full hydration.
Figure 6.7: $T_1$ distributions of solid and liquid protons at different moisture contents. Plots on the left-hand side are from a white matter sample and plots on the right-hand side are from a grey matter sample. (Note that the amplitude of the fully hydrated sample has been reduced by a factor of 2.)
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Figure 6.8: Plot of the saturation recovery curve for a white and grey matter sample. The curves shown come from the equation $S(t) = M_0(1 - e^{-t/TR})$. 
## Table 6.2: $T_s$ measured in Goldman-Shen cross relaxation experiment for white and grey matter samples. Moisture contents are included for brain 3 samples. Calculated cross relaxation times are also included.

<table>
<thead>
<tr>
<th>Sample (MC%)</th>
<th>White $T_s$ (ms)</th>
<th>$T_{cr}^m$ (ms)</th>
<th>$T_{cr}^i$ (ms)</th>
<th>Grey $T_s$ (ms)</th>
<th>$T_{cr}^i$ (ms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1-2</td>
<td>18.9</td>
<td>37.2</td>
<td>144</td>
<td>1-1</td>
<td>18.5</td>
</tr>
<tr>
<td>1-6</td>
<td>21.3</td>
<td>41.9</td>
<td>165</td>
<td>3-4 (77.0)</td>
<td>22.9</td>
</tr>
<tr>
<td>3-1 (71.0)</td>
<td>20.2</td>
<td>52.6</td>
<td>174</td>
<td>3-4 (52.8)</td>
<td>13.7</td>
</tr>
<tr>
<td>3-2 (73.2)</td>
<td>24.6</td>
<td>48.7</td>
<td>212</td>
<td>3-5 (85.4)</td>
<td>33.1</td>
</tr>
<tr>
<td>3-2 (68.2)</td>
<td>19.8</td>
<td>35.6</td>
<td>141</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-2 (59.9)</td>
<td>16.8</td>
<td>27.2</td>
<td>105</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-2 (37.7)</td>
<td>9.8</td>
<td>12.8</td>
<td>36.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 (72.0)</td>
<td>25.5</td>
<td>58.9</td>
<td>230</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3-6 (36.0)</td>
<td>10.1</td>
<td>18.4</td>
<td>58.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Assumed: one between the myelin water and solid myelin pool ($T_{cr}^m$) and the other between the intra/extracellular water and non-myelin molecule pool ($T_{cr}^i$). For the first case, $P_s^m$ was calculated by:

$$P_s^m = \frac{S_m}{S_m + S_{mw}}.$$  \hspace{1cm} (6.1)

A small normalisation correction was applied to $S_{mw}$ since the CPMG and FID experiments did not yield exactly the same total mobile intensities. This normalisation consisted of multiplying $S_{mw}$ by the ratio of the total FID mobile signal and the total CPMG signal at $t=0$. In the second case, $P_s$ was calculated by:

$$P_s^{nm} = \frac{S_{nm}}{S_{nm} + S_{iw}}.$$ \hspace{1cm} (6.2)

The same normalisation correction was applied to $S_{iw}$. $T_{cr}$ was then calculated as the ratio of $T_s$ to $P_s$ for each case. $T_s$ and both cross relaxation times for all samples are listed in Table 6.2. As the brain was dehydrated, both cross relaxation times decreased.
Figure 6.9: A plot of the solid signal recovery in a cross relaxation experiment. A white and grey sample are presented. The cross relaxation fits to these data are also shown.
Chapter 6. Relaxation Measurements of Bovine Brain using Magnetic Resonance

White

- Diffusion (very slow)

- Cross relaxation ($T_{cr}^m$)

- Myelin Water ($S_{mw,T_1^{mw},T_2^{mw}}$)

- Intra/Extra Cellular Water ($S_{iw,T_1^{iw},T_2^{iw}}$)

- Cross relaxation ($T_{cr}^l$)

- Myelin Molecules ($S_{m,T_1^{m},T_2^{m}}$)

- Non-myelin Molecules ($S_{nm,T_1^{nm},T_2^{nm}}$)

Grey

- Cross relaxation ($T_{cr}^l$)

- Intra/Extra Cellular Water ($S_{iw,T_1^{iw},T_2^{iw}}$)

- Non-myelin Molecules ($S_{nm,T_1^{nm},T_2^{nm}}$)

Figure 6.10: Model of the different proton pools in bovine brain
6.4.5 \( T_1 - T_2 \) Dependence

A plot of the \( T_1 - T_2 \) dependence of a white matter sample is shown in Figure 6.11. From this experiment, water with a short \( T_1 \) was also found to have a short \( T_2 \). There was only one off-diagonal element found in one sample and its intensity was very low. The 3-D plots were divided into two regions, one consisting of peaks at short \( T_1 \) (< 750 ms) and \( T_2 \) (< 40 ms) and the other of peaks at long \( T_1 \) (> 800 ms) and \( T_2 \) (> 40 ms), and the ratio of their intensities averaged to 20%.

6.4.6 Cross-\( T_2 \) Dependence

Plots of cross-\( T_2 \) relaxation are shown in Figure 6.12A and B. Plots of \( T_1 - T_2 \) relaxation are also shown as a comparison. The cross-\( T_2 \) decay curves arising from the myelin water in hydrated white matter were fit to an exponential with an average relaxation value, \( T_{relax}^{m} \), of 36 ms. In contrast, the relaxation value for the \( T_1 - T_2 \) decay curves from the myelin water gave a value of 371 ms. The cross-\( T_2 \) decay curves for the intra/extracellular water from hydrated white matter were found to have an average \( T_{relax}^{i} = 178 \) ms. For the \( T_1 - T_2 \) decay curves, the intra/extracellular water was found to have an average \( T_{relax} = 797 \) ms. In grey matter, the cross-\( T_2 \) decay curve for the myelin water could not be fit to an exponential since there was only a small short \( T_2 \) component present. The relaxation time for the intra/extracellular water was found to be 175 ms which was the same as white matter. Measured relaxation rates in the cross-\( T_2 \) experiment and the calculated cross relaxation times for each water pool are shown in Table 6.3 for some samples. The \( T_s \) values presented in the table were calculated from the \( P_m, P_{nm} \) and \( T_w \)'s measured in this experiment.
Figure 6.11: A plot of the $T_1$-$T_2$ dependence of a white matter sample. Plot A shows a 3D view of the data while plot B shows a bird's eye view of $T_1$ versus $T_2$. 
Figure 6.12: Plots showing the cross relaxation dependence of myelin and intra/extracellular water in white matter (closed circles). Plot A represents the myelin water component and plot B the intra/extracellular water. Exponential fits to each data set are also shown. The data from the $T_1$ dependence of myelin and intra/extracellular water are shown for reference (open circles). Note, only the first 200 ms of the $T_1$ and $T_2$ data are shown.
Table 6.3: Measured $T_w$ times for both water pools using the cross-$T_2$ experiment. The calculated cross relaxation are also included for some white and grey samples. The hydrated sample is an average over the three fully hydrated white matter samples from Brain 3. Standard errors are shown in parentheses.
6.5 Discussion

The main difference between white and grey matter is myelin which makes up about 50% of the dry mass of white matter tissue [6]. Therefore, the relative amount of solid signal is expected to be twice as big in white matter as in grey matter. This was found to be the case for both the FID experiment and the T1 experiment where solid and liquid signals were separated (Figures 6.1 and ??). Water content for human white and grey matter have been found to be 70% and 80% respectively [6]. The average water content of fully hydrated grey matter samples was larger than that of white matter although the absolute values are not necessarily accurate since this was an in vitro experiment. The water content of myelin is 40% which gives us an estimate of the myelin water percentage in white matter being 16%. This is fairly close to the average of 14.3% measured in bovine white matter. The estimated proton densities found for both white and grey matter fall within the proton densities of its constituents: lipids (0.13 gH/gLipid) and proteins (0.04-0.06 gH/gProtein).

The mobility or orientational order of the solid protons is measured using the second moment. Both white and grey matter had similar second moments indicating that the amount of order in both tissues is similar. The second moment of phospholipid bilayers has been previously measured as $5 \times 10^8 \text{s}^{-2}$ for the liquid crystal phase and $3.5 \times 10^9 \text{s}^{-2}$ for the gel phase [83] and typical proteins as $5 \times 10^9 \text{s}^{-2}$ [84]. The second moment of fully hydrated bovine brain tissue falls between the second moment of liquid crystalline phospholipids and proteins.

The T2 distribution generally showed four peaks for fully hydrated samples. In white matter, two of the peaks were centred about 100 ms and may represent separation of the intracellular and extracellular fluid. All peaks below 50 ms were attributed to myelin water due to the short T2 relaxation time. Although the shape of the T2 distribution was
broader for the $\tau = 100 \, \mu s$ experiment than the $\tau = 200$ or $400 \, \mu s$, the $T_2$ times between experiments were indistinguishable and the myelin water percentages were similar (24.1, 22.6 and 18.1 for the 100, 200 and 400 $\mu s$ experiments respectively). When the samples were dehydrated, the myelin water percentage stayed fairly constant indicating that all the water in the sample was decreasing at the same rate. This was surprising since it was expected that water in the intra/extracellular spaces would evaporate before more tightly bound water trapped between myelin bilayers.

The $T_1$ of both the solid and liquid components of grey matter coincided at approximately 1.2 s. In white matter, the $T_1$ of solid was measured at about 500 ms while the liquid peak was at 800 ms. The similarity in grey $T_1$s is thought to arise from averaging between the intra/extracellular water pool and the non-myelin macromolecular pool (Figure 6.10). In white matter, only myelin water is in close contact with the solid and therefore much less averaging must take place. This allows differences in the $T_1$ values for the solid and mobile pools. A difference in $T_1$ for the two water pools was seen by another group [80] but no explanation as to the mechanism was given. The single $T_1$ value found for the two liquid pools in this work is attributed to the inability of NNLS to separate peaks which are within a factor of two in relaxation time. No cross peaks were found between the $T_1$ and $T_2$ components which indicated that there was no exchange on the timescale of 1 s between the two water pools. This was different than results found from in vivo human brain where a single $T_1$ component indicated mixing between the two pools. It should be noted however that these experiments were carried out at room temperature and that the rate of diffusion would be increased at physiological temperatures.

Since there was no mixing of the two water pools, each cross relaxation time, $T_{cr}^m$ and $T_{cr}^n$, was calculated for the separate proton pools (Figure 6.10). In the case of the solid signal, half the intensity was attributed to myelin and half to other macromolecules.
Chapter 6. Relaxation Measurements of Bovine Brain using Magnetic Resonance

since myelin comprises about 50% of the dry weight of white matter [6]. Two different relaxation times, $T_{wm}$ and $T_{wm}$, were measured from the cross-$T_2$ dependence. These values as well as $P_m$ and $P_{nm}$ were used to calculate the expected $T_{wm}^m$ and $T_{wm}^{nm}$ (equal to 29 ms and 22.5 ms respectively). Since both values were quite close, only one $T_s$ time should have been measurable in the Goldman-Shen cross relaxation experiment. The individual cross relaxation times, $T_{wm}^m$ and $T_{wm}^{nm}$, were calculated from the single $T_s$ and the probability of protons being in either pool, $P_m$ and $P_{nm}$. In grey matter, it was assumed that all the solid signal belonged to the non-myelin molecule pool and all the mobile signal to intra/extracellular water. The expected $T_{wm}^{nm}$ was calculated and found to be 18 ms. This was slightly different than the measured value of 33 ms. The reason for this discrepancy is unknown. Cross relaxation times have been measured in wood and found to be 1 ms in red cedar and 4 ms in hemlock [85]. These are an order of magnitude smaller than the cross relaxation time in myelin probably due to the water in the cell wall having much greater interactions with the solid protons than water in myelin.

The decay of the solid is governed by dipolar interactions between protons which causes the spins to dephase quickly. Cross relaxation between the solid and myelin water protons contributes to the $T_2$ relaxation time of the mobile signal. Therefore, $T_w$ is an upper limit for the $T_2$ time of mobile protons, i.e.

$$\frac{1}{T_2} \geq \frac{1}{T_w} = \frac{1}{P_w T_{cr}}$$

If we assume that cross relaxation is the only mechanism responsible for $T_2$ relaxation in grey matter and that all the water is in contact with all the solid, then the expected $T_2$ time is about 175 ms. Since the measured value was about 100 ms, cross relaxation is not the only mechanism for $T_2$ relaxation. In white matter, the assumption was that the main source of cross relaxation was between the myelin water and the myelin. If we then calculate the expected $T_2$ for myelin water, the result is around 37 ms. This is slightly
larger than the measured value of about 20 ms. Therefore, mechanisms other than cross relaxation also affect the $T_2$ relaxation times in white and grey matter.

6.6 Conclusion

A model for $T_1$ and $T_2$ relaxation of water in bovine brain has been proposed which incorporates 4 proton pools: intra/extracellular water, myelin water, non-myelin macromolecules and myelin macromolecules. During the course of an NMR experiment ($<1$ s), the two water pools do not exchange. There is, however, rapid exchange between the myelin water and myelin pools ($T_{cr}^m = 66$ ms) and slightly slower exchange between the intra/extracellular and non-myelin pools ($T_{cr}^i = 193$ ms). The myelin water protons are found to have a short $T_2$ value partly due to cross relaxation with the myelin. Although a simple $T_1$ experiment is not able to distinguish the two water pools, a $T_1$-$T_2$ experiment is able to separate the myelin water based on its short $T_1$ and $T_2$. 
Chapter 7

Diffusion model of $T_2$ and $T_1$ relaxation in two brain water pools

7.1 Summary

A diffusion model of myelin water and intra/extracellular water was used to simulate $T_1$ and $T_2$ relaxation data from white matter in human brain. Relaxation times and amplitudes were calculated from models where different parameters including diffusion coefficients, and cell sizes were varied. Generally, changes in the intra/extracellular pool parameters had little effect on the relaxation while changes to the myelin pool parameters caused all relaxation times to vary. The diffusion coefficient of the myelin water pool was estimated to be about $0.0002 \, \mu m^2/ ms$. Increases in the myelin thickness and axonal diameter (in the same proportion) caused increases in the myelin water percentage and $T_2$ relaxation time of the myelin water component which could account for the experimental differences in myelin water percentage between different white matter structures.

7.2 Introduction

Relaxation in biological tissue is multi-exponential with time constants shorter than those found in bulk water. Mechanisms describing the relaxation have been proposed incorporating diffusion between different proton pools. A preliminary model by Brownstein and Tarr [86] involved a single water pool which relaxed due to interaction with strongly relaxing surfaces. This model worked well for certain biological cells such as wood [87] but did not take into account the role of cytoplasmic components of the cell or the presence
of different water pools. Belton and Hills [88] expanded the diffusion model to include two water pools separated by a permeable or semi-permeable membrane. Their approach used the Laplace transform method to obtain an analytical solution for diffusion.

In human brain, two water pools (myelin water and intra/extracellular water) have been suggested on the basis of $T_2$ relaxation experiments [16, 17]. The intra/extracellular water pools are combined into one since they can not be distinguished on the basis of in vivo relaxation measurements. Exchange between the myelin water and intra/extracellular water is thought to occur within 500 ms since $T_1$ relaxation is mono-exponential [17] and MT delay measurements show averaging at long times (see Chapter 5). The likely mechanism for this exchange is diffusion.

In this chapter, a diffusion model is applied to the two water pools, one in a cylindrical shell and the other inside the cylinder, in order to simulate in vivo $T_2$ and $T_1$ relaxation data (Figure 7.1). The diffusion-Bloch equation is solved numerically for cylindrical coordinates since axons are cylindrical in nature. Diffusion coefficients, cell radii and $T_2$ and $T_1$ relaxation times are input into the model and the measured output consists of $T_2$ and $T_1$ relaxation times and amplitudes.

7.3 Numerical Methods

The effect of diffusion on the magnetization, $M(r, t)$, of the two water pools can be described by the diffusion-Bloch equation

$$\frac{\partial M(r, t)}{\partial t} = D \frac{\partial^2}{\partial r^2} + \frac{1}{r} \frac{\partial}{\partial r} M(r, t) - \frac{M(r, t)}{T_{2,1}}$$  \hspace{1cm} (7.1)

where $D$ is the diffusion coefficient of the water. This equation was discretised (in time steps $\Delta t$ and radius steps $\Delta r$) using the Crank-Nicholson [89] method to obtain

$$\frac{M_{r,t+1} - M_{r,t}}{\Delta t} = D \left[ \frac{M_{r+1,t} - 2M_{r,t} + M_{r-1,t}}{(\Delta r)^2} + \frac{1}{r\Delta r} \frac{M_{r+1,t} - M_{r-1,t}}{2\Delta r} \right] - \frac{M_{r,t}}{T_2} \hspace{1cm} (7.2)$$
Figure 7.1: A schematic showing the two different cylindrical pools of water in the brain and their associated parameters.
where *r* and *t* are the radius and time interval indices. This equation can be reduced to the form

\[
M_{r,t+1} = [F - G_r]M_{r-1,t} + [1 - 2F - \frac{\Delta t}{T_2}]M_{r,t} + [F + G_r]M_{r+1,t}
\]  

(7.3)

where

\[
F = \frac{D\Delta t}{(\Delta r)^2}
\]  

(7.4)

and

\[
G_r = \frac{D\Delta t}{2r(\Delta r)^2}
\]  

(7.5)

Other definitions in equation 7.3 are

\[
D = \begin{cases} 
D_i; & 0 \leq r \leq a \\
D_m; & a < r \leq b 
\end{cases}
\]  

(7.6)

\[
T_2 = \begin{cases} 
T_{2i}; & 0 \leq r \leq a \\
T_{2m}; & a < r \leq b 
\end{cases}
\]  

(7.7)

where *a* is the position of intra/extracellular water and myelin water boundary and *b* is the outer boundary of the region (Figure 7.1).

The initial magnetization (at *t*=0) is defined as

\[
M_{r,0} = \begin{cases} 
M_o; & 0 \leq r \leq a \\
KM_o; & a < r \leq b 
\end{cases}
\]  

(7.8)

where *K* is the partition coefficient between the concentrations of the two water pools. The solution is symmetric and continuous about *r* = 0 which implies that \(\frac{\partial M}{\partial r}|_0 = 0\).

Using the forward Euler method of discretisation, we can obtain expressions for \(M_{r+1}\). At the other boundary, *r* = *b*, we again impose symmetry and \(\frac{\partial M}{\partial r}|_b = 0\). Using a backward Euler method of discretisation, we can obtain expressions for \(M_{r-1}\). At the boundary, *r* = *a*, we must invoke Fick's Law or continuity of flux which gives

\[
-K^{2/3}D_i \frac{\partial M}{\partial r}|_a^- = -D_m \frac{\partial M}{\partial r}|_a^+
\]  

(7.9)
Since the density of water is different on either side of the boundary and density is related to $K$, a $K^{2/3}$ term was added to the equation in order to correct for flux through different unit areas. This can be discretised using a forward Euler method to get

$$-K^{2/3} D_t \frac{M_{a,t} - M_{a-1,t}}{\Delta r} = -D_m \frac{W_{a+1,t} - W_{a,t}}{\Delta r}$$

(7.10)

$$-K^{2/3} D_t \frac{M_{a,t+1} - M_{a-1,t+1}}{\Delta r} = -D_m \frac{W_{a+1,t+1} - W_{a,t+1}}{\Delta r}$$

(7.11)

where the magnetizations are defined as

$$M_{r,t} = \begin{cases} M(r,t); & 0 \leq r \leq a \\ W(r,t); & a < r \leq b \end{cases}$$

(7.12)

Again at the boundary $r = a$, the partition balance equation gives

$$W_{a,t+1} = KM_{a,t+1}.$$  

(7.13)

where the $K$ is included to account for the different densities of water on either side of the boundary. When equations 7.10, 7.11 and 7.13 are combined, the resulting expression is a forward "marching" in time equation for the magnetization at $r = a$.

The stability condition for this equation is $\Delta t < \frac{(\Delta r)^2}{2D_t}$.

### 7.4 Numerical Applications

Most of the parameters which are involved in the numerical simulation are unknown for brain. Therefore, the best initial guesses were used and then each input parameter was varied. Myelin water makes up 16% of the total water in white matter whereas intra/extracellular water is 84%. The partition coefficient can be calculated from these water contents, the model cell radii, $a$ and $b$, and the expected magnetization such that

$$K = \frac{0.16}{0.84} \frac{a^2 M_o}{(b^2 - a^2)M_o}$$

(7.14)
where $M_o$ is the initial magnetization as defined in equation 7.8. Data on nerve axon diameters were very hard to obtain but one paper gave values for the corpus callosum [90]. This was used to estimate the initial radii, $a$ and $b$, to be 1.0 $\mu$m and 1.2 $\mu$m respectively which corresponds to a $K$ of 0.4. This $K$ is equivalent to the moisture content of the myelin water pool which has been measured at 40% [6].

The diffusion coefficient of bulk water at room temperature is about 2.0 $\mu$m$^2$/ms. The diffusion coefficient of the intra/extracellular water was guessed to be about 100 times less than free water, giving $D_i = 0.02 \mu$m$^2$/ms. Using the diffusion equation, $D = r^2/2t$ and the above cell radii, the diffusion coefficient of myelin water was estimated to be $D_m = 0.00002 \mu$m$^2$/ms. This value of $D_m$ results in minimal mixing of the two water pools in 100 ms and partial mixing in 500 ms which models MRI $T_2$ and $T_1$ experiments.

Also estimated were $T_{2m} = 20$ ms and $T_{2i} = 200$ ms from MRI $T_2$ relaxation experiments. The $T_1$ relaxation times were set to $T_{1m} = 200$ ms and $T_{1i} = 800$ ms since myelin water was believed to have a lower value than intra/extracellular water.

The step sizes for the $T_2$ simulations were $\Delta r = 0.005 \mu$m and $\Delta t = 0.00005$ ms. Enough time steps were taken to simulate a 32 echo decay curve. For $T_1$ simulations, $\Delta r = 0.005 \mu$m and $\Delta t = 0.0005$ ms. Eight data points were collected at times of 10, 20, 50, 100, 200, 500, 1000 and 2000 ms to produce a decay curve. Both the $T_2$ and $T_1$ decay curves were fit to bi-exponential models to obtain relaxation times and amplitudes for each pool. The fitting algorithm chose all pairs of $T_1$ and $T_2$ relaxation times from an input of 100 possible relaxation times and, using an NNLS algorithm, output the pair of relaxation times and amplitudes which gave the lowest $\chi^2$. 

7.5 $T_2$ simulations

Variations in radii were done with a constant $K = 0.4$ but changing $a$ and $b$ such that $a/b$ remained constant. The effect on each pool is seen in Figure 7.2. $T_{2i}$ was greatly reduced by decreases in the myelin thickness but $T_{2m}$ was only reduced at a very small myelin thickness. The amplitude of each $T_2$ component was fairly constant except at small myelin thickness which caused the amplitude of the myelin water pool to decrease and the intra/extracellular water pool to increase.

Figure 7.2: A plot showing the effect of changing the cell radii and the myelin thickness on the $T_2$ and $T_1$ relaxation times and amplitudes. The cell radius and myelin thickness were increased proportionally in order to keep $K$ constant at 0.4. The amplitude of the relaxation components are shown in % for $T_2$ and fractions for $T_1$.

The effect of changing the initial $T_{2i}$ or $T_{2m}$ is shown in the lower half of Figure 7.3. Decreasing the initial $T_{2i}$ only decreased the $T_{2i}$ relaxation time. Decreasing the initial
Table 7.1: Myelin water percentages from simulations with different axon diameters and myelin thickness are shown and compared to experimentally measured myelin water percentages for different white matter structures. The parameters used were $D_i = 0.02$ and $D_m = 0.0002 \mu m^2/ms$, and $T_{2i} = 200$ and $T_{2m} = 30$ ms. The input myelin water percentage was 15% for all cases.

$T_{2m}$ caused a decrease in both $T_{2i}$ and $T_{2m}$. The amplitudes were fairly constant for both cases.

The diffusion coefficients of each pool were varied separately and the effect is shown in the lower half of Figure 7.4. Changing $D_i$ caused slight fluctuations in the relaxation time of each pool. When $D_m$ was increased, $T_{2i}$ decreased dramatically while $T_{2m}$ decreased slightly. However, at high values of $D_m$, the amplitude of the myelin water component became 0.

Since the amplitude of the myelin water component was very dependent on the axon diameter and the myelin thickness, these two parameters were varied in order to obtain myelin water percentages equal to the different white matter structures in chapter 4. Results are shown in Table 7.1. For these simulations, $D_i = 0.02$ and $D_m = 0.0002 \mu m^2/ms$ and $T_{2i} = 200$ and $T_{2m} = 30$ ms which resulted in better matches to the experimental data.
Chapter 7. Diffusion model of $T_2$ and $T_1$ relaxation in two brain water pools

Figure 7.3: A plot showing the effect of changing the initial $T_1$ (upper) and $T_2$ (lower) on the simulated $T_2$ and $T_1$ relaxation times and amplitudes. On the left, $T_i$ was varied and on the right, $T_m$ changed. The amplitude of the relaxation components are shown in % for $T_2$ and fractions for $T_1$. 
Chapter 7. Diffusion model of $T_2$ and $T_1$ relaxation in two brain water pools

Figure 7.4: A plot showing the effect of changing the diffusion coefficient on the $T_1$ (upper) and $T_2$ (lower) relaxation times and amplitudes. On the left, $D_i$ was varied and on the right, $D_m$ was changed. The amplitude of the relaxation components are shown in % for $T_2$ and fractions for $T_1$. 
7.6 \( T_1 \) simulations

As with the \( T_2 \) simulations, \( a \) and \( b \) radii were varied with \( a/b \) constant. The effect on each pool is seen in Figure 7.2. Both \( T_{1u} \) and \( T_{1m} \) were reduced as the myelin thickness was reduced except at very small thickness when \( T_{1m} \) increased dramatically. The amplitude of the myelin water pool paralleled the behaviour of \( T_{1m} \).

The effect of changing the initial \( T_{1u} \) or \( T_{1m} \) is shown in the top half of Figure 7.3. Decreasing the initial \( T_{1u} \) only decreased the \( T_{1u} \) relaxation time. Decreasing the initial \( T_{1m} \) caused a decrease in both \( T_{1u} \) and \( T_{1m} \). The amplitudes were fairly constant for the first case but the amplitude of the myelin water pool decreased and then increased for the second case.

The diffusion coefficients of each pool were varied separately and the effect is shown in the top half of Figures 7.4. Increasing \( D_i \) caused slight decreases in the relaxation time of each pool. The amplitude of the myelin water pool was also slightly decreased. When \( D_m \) was increased, \( T_{2i} \) decreased while \( T_{2m} \) increased resulting in a single relaxation time. The amplitudes of each component also varied.

7.7 Discussion

The goal of these simulations was to examine the trends in \( T_1 \) and \( T_2 \) relaxation and amplitudes by varying parameters which are not available experimentally. Although myelin is composed of multiple bilayers, the model treated the whole structure as uniform with the parameters \( D \), \( T_1 \) and \( T_2 \) taking into account the bilayers. Also, since intracellular and extracellular water pools are not separable from \textit{in vivo} \( T_2 \) relaxation measurements, both pools were combined into the intracellular pool. Certain parameters between the intracellular and extracellular water pools are expected to be slightly different, such as
diffusion coefficients, but since only one compartment can be measured in vivo, these differences were averaged into one pool. Also, the extracellular water pool is approximately five times smaller than the intracellular water pool and therefore would have a smaller effect on the results. Consequently, this model is only a first approximation of diffusion in white matter.

One of the main assumptions when measuring myelin water percentages in vivo was that the amplitude of the short $T_2$ component was equal to or at least related to the amount of water in the myelin water pool. With the simulations, we were able to test this hypothesis. When either $T_{1i}$ or $T_{2i}$ was changed, there was no change in the amplitude of either pool. Therefore, any pathological problem which may cause a change in the $T_1$ or $T_2$ of the intra/extracellular water should not affect the myelin water pool. Similarly, when $T_{2m}$ was increased there was no change in the amplitudes of the $T_2$ components. It should be noted that the expected amplitude for the myelin water $T_2$ component was 15% and not the 7% output by the simulation.

From the simulations, it was obvious that changing the properties of the myelin water pool influenced the relaxation times and amplitudes to a much greater extent than did varying the intra/extracellular water pool parameters. This was expected since the myelin water pool acted like a relaxation sink for water and therefore has a greater influence on the intra/extracellular water pool. This was further supported by increases in the myelin water diffusion coefficient causing decreases in the intra/extracellular water relaxation time indicating more mixing of the two pools.

Evidence for partial mixing of the water pools was found at diffusion coefficients of $D_i = 0.02$ and $D_m = 0.00002 \ \mu m^2/ms$ since changes in $T_{2m}$ and $T_{1m}$ caused $T_{2i}$ and $T_{1i}$ to also change. This is likely due to exchange between the water pools averaging the relaxation times. However, at this value of $D_m$, two $T_1$ relaxation times were still discernible which was not consistent with experiment. A $D_m$ of 0.0002 $\mu m^2/ms$ caused
the two water pools to become completely averaged. This larger value of \( D_m \) also caused the amplitude of the short \( T_2 \) component to decrease slightly indicating that there was some exchange within the \( T_2 \) timescale. Therefore, short \( T_2 \) amplitudes measured experimentally may be slightly lower than the actual values. The "best guess" value of \( D_i \) used in the simulations was lower than that found in the literature of 0.5 \( \mu m^2/\text{ms} \) [91] although this was not for human nerve. Fortunately, changes in \( D_i \) had little to no effect on output relaxation times and amplitudes and therefore these results should still be valid for larger values of \( D_i \). Future simulations will incorporate a more realistic diffusion coefficient. Exchange was also seen with small values for the myelin thickness which caused large decreases in \( T_{2i} \), suggesting that a larger portion of the myelin water was able to interact with the intra/extracellular water. In \( T_1 \) simulations, the small myelin thickness caused the \( T_1 \)s of the two water pools to become similar, again indicating averaging.

Since myelin is fairly impermeable to water, a possible mechanism for myelin water exchange is that it diffuses circularly around the rings of the bilayer until it reaches either the intra or extracellular space. This time to travel around the bilayers scales as the square of the number of layers. If we assume that myelin water has a diffusion coefficient equal to free water (2.0 \( \mu m^2/\text{ms} \)) in between the bilayers and the radius of the cell is 1 \( \mu m \), then the time to travel from the inside to the outside of the myelin (about 10 bilayers thick) is about 1 s. This may account for the averaging found within the \( T_1 \) timescale but not the \( T_2 \) timescale in human brain.

A very exciting result seen from the simulations is that the \( T_2 \) of the intra/extracellular water changes depending on the thickness of the myelin and the axonal diameter. Smaller axons with thinner myelin sheaths were expected to have shorter \( T_{2i} \). Also, from Table 7.1, it can be seen that smaller axons with thinner myelin also have smaller apparent myelin water percentages even though the input myelin water percentages was equal to 15% for all cases. From experiment, it was found that different white matter structures
have different measured myelin water percentages and can be ranked accordingly. This ranking was preserved when looking at the geometric mean $T_2$ [17]. Therefore, different myelin water percentages and different $T_2$ values measured in vivo may be a consequence of different sizes of axons and myelin thickness. It should be pointed out that these simulations involved a model where the ratio of the axon diameter to myelin thickness remained fixed which is likely not the case in brain. From the simulations, we have found that the myelin water percentage is related not only to the amount of myelin water but also to the morphology of the axons as well as parameters such as the diffusion coefficient.

### 7.8 Concluding Remarks

Relaxation in human white matter can be modelled as diffusion between two water pools. Changes to the diffusion coefficient and relaxation time of the myelin water pool can cause dramatic differences in the relaxation times and amplitudes of both water pools. The diffusion coefficient of the myelin water pool was estimated to be 0.0002 $\mu m^2/\text{ms}$ since this value caused complete averaging of the two water pools during the $T_1$ timescale but not the $T_2$ timescale. The diffusion coefficient of the intra/extracellular water pool could not be estimated since changes in its value had little effect on the output relaxation times and amplitudes. A proportional increase in the diameter of the axon and the myelin thickness also increases the myelin water percentage which may account for the differences in myelin water percentage found between different white matter structures. The $T_2$ relaxation time of the myelin water component also increased as the diameter of the axon and the myelin thickness increased.
8.1 This work

In this thesis, human brain was examined using MRI and bovine brain was examined using $^1$H-NMR. Measurements that were supposedly related to myelin content were explored in both normal and multiple sclerosis brain. Since there are two water pools in the brain, the MT effect on each was investigated. Finally, experiments in bovine brain were done to explore how different proton pools interacted and over what timescale these interactions occurred. Simulations were also carried out to examine how different water pool parameters could affect the amplitude and value of relaxation rates.

Magnetization transfer ratios and myelin water percentages were uncorrelated in white and grey matter from both normal volunteers and MS patients. In lesions, there was a small ($R=0.5, P=0.005$) but significant correlation. The lack of strong correlation was surprising since it indicates that the methods can not both be proportional to myelin contents.

The effect of MT is different for each brain water pool with the short $T_2$ component being affected significantly more than the long $T_2$ component ($P<0.00001$ to $P<0.04$). This finding helps strengthen the argument that the short $T_2$ component is myelin water which would be closely associated with myelin molecules. With different types of MT pulses, different amounts of direct saturation were seen and the amount of direct saturation appeared to be proportional to the amount of myelin water. This allowed
the binomial MT pulse (which produced large amounts of direct saturation) to differentiate between different white matter structures while the sinc MT pulse (small direct saturation effect) did not.

In bovine brain white matter, a 4-pool model with cross relaxation between the myelin and myelin water (named myelin pools) and the intra/extracellular water and non-myelin molecules (named non-myelin pools) was found to represent the relaxation data. Surprisingly, no exchange between the two water pools was found within the timescale of our measurements (within 1 s). In grey matter, a 2-pool model was invoked which only included the intra/extracellular water and the non-myelin molecules. The cross relaxation time between the myelin water and myelin was found to be 66 ms and between intra/extracellular water and non-myelin to be 193 ms for fully hydrated white matter. For grey matter, the cross relaxation time was found to be about 200 ms.

One of the main differences between the bovine and human brains was the rate of exchange between the two water pools. As stated earlier, in bovine brain, there was no exchange between these two pools over the timescale of our measurement. In human brain, exchange was found within about 500 ms. Since the bovine brain was removed from the cow before imaging, it is possible that these differences are due to in vivo versus in vitro studies rather than differences between the brains.

Simulations which modelled the exchange between the water pools in human brain MRI experiments through diffusion showed that relaxation times were highly dependent on the diffusion coefficient of the myelin water pool but not that of the intra/extracellular water pool. Differences in the size of the myelin cylinder compared to the axon cylinder caused changes in the relaxation time of the intra/extracellular water pool but not much change to the myelin water pool. The amplitude of the myelin water component was dependent on the axon diameter and myelin thickness. Therefore, different white matter structures may have different myelin water percentages due to different axon cell sizes.
8.2 On-going and future work

Currently, a serial study has been started which will examine lesions over the course of one year using MT, $T_2$ relaxation and $T_1$ relaxation. The patients have at least one enhancing lesion which is viewed by all three techniques. $T_1$ times will be used to correct for relaxation during the scan and therefore accurate water contents can be obtained. Comparisons are planned between all techniques as well as monitoring the progression of lesions over time.

Further experiments using $T_2$ relaxation with an MT prepulse will be done in order to get better statistical numbers. In particular, more normal volunteers will be examined using different frequency offsets and delay times between the MT pulse and the initiation of the $T_2$ relaxation sequence. A time constant associated with the rate of exchange between the two water pools may be obtained from the delay experiment.

Since the assumption that myelin water is water trapped between myelin bilayers has not been completely substantiated, biochemical analyses of bovine brain white matter samples will be done in conjunction with $^1$H-NMR measurements. It is hoped that a correlation between biochemically measured myelin content and the myelin water percentage will be found.

Finally, fixed brains have been obtained from a number of deceased MS patients. These brains will be scanned using the $T_2$ relaxation and MT sequences and then cut through the appropriate slice in order to perform myelin staining. The pathology of lesions found in the slice will be compared to the MRI results to see if correlations can be found. This may ultimately determine which MRI method best represents the state of the lesion as well as giving correlations between the pathology and magnetization transfer ratios and myelin water percentages.
Bibliography


[88] P. S. Belton and B. P. Hills. The effects of diffusive exchange in heterogeneous


Appendix A

Source Code for $T_2$ Simulations

c This program will calculate the U matrix from the set of
c diffusion equations in a cylinder
c
c calls TRIDIAG
c
PROGRAM EULER_EQN
c
real*8 a,b
real*8 Df,Dm,T2f,T2m,Mo,k
real*8 deltar,deltat
integer nr,nt,counter,tester
real*8 m(10000),n(10000),p(10000),q(10000)
real*8 Ff,Gf(10000),Fm,Gm(10000)
real*8 sum,sump,sumq,sumtotal
real*8 decayp(32),decayq(32),decayt(32)
character*50 filename

c Input the parameters
c
read(5,*)filename
write(6,*) 'Enter Filename ',filename
read(5,*) a,b
write(6,*) 'Input radius of axon and axon+myelin:',a,b
read(5,*) Df,Dm
write(6,*) 'Input diffusion constant of area 1 and area 2',Df,Dm
read(5,*) T2f,T2m
write(6,*) 'Input T2 of area 1 and area 2',T2f,T2m
read(5,*) Mo
write(6,*) 'Initial magnetisation',Mo
read(5,*) k
write(6,*) 'Partition coefficient',k
read(5,*) deltar
write(6,*) 'radius step size',deltar
read(5,*) deltat
write(6,*) 'time step size',deltat
read(5,*) nt
write(6,*) 'Number of time points',nt
nr=IIDINT(b/deltar)

c Calculate F and G functions
c
Ff=Df*deltat/(deltar**2)
Fm=Dm*deltat/(deltar**2)
c write(6,*)'Ff and Fm',Ff,Fm
Gf(1)=0
c open(unit=Gstuff,File='Gstuff.dat',status='new')
Do 10 i=2,nr+1
    if (i .le. IIDINT(a/deltar+1)) Gf(i)=Df*deltat/(2*(i-1)*
        deltar**2)
    if (i .ge. IIDINT(a/deltar+1)) Gm(i)=Dm*deltat/(2*(i-1)*
        deltar**2)
c write(Gstuff,*)i,' Gf and Gm',Gf(i),Gm(i)
10 Continue
c Close(unit=Gstuff)
c
c Define initial t and m matrices
c
Do 15 i=1,nr+1
    if (i .le. IIDINT(a/deltar+1)) m(i)=Mo
    if (i .ge. IIDINT(a/deltar+1)) n(i)=K*Mo
15 Continue
c
c Use Trapezoidal Rule to add up magnetization
c
da=IIDINT(a/deltar+1)
sump=0
sump=deltar**2*(0*m(1)+nr*m(nr+1))/2
sumq=0
sumq=deltar**2*(0*n(1)+nr*n(nr+1))/2
Do 45 i=2,nr
    sump=sump+(i-1)*deltar**2*m(i)
    sumq=sumq+(i-1)*deltar**2*n(i)
Appendix A. Source Code for $T_2$ Simulations

45      Continue
       sumtotal=sump+sumq
       write(6,*) j, sump, sumq, sumtotal
       
       counter=0
       open(unit=IData, File=filename, status='new')
       npoints=32
       stdev=1.0
       write(IData,*) npoints, stdev
       
       open(unit=Test, File='test.dat', status='new')
       
       Do 50 j=1, nt
       write(6,*) j
       
       Do 20 i=1, nr+1
       if (i .eq. 1) then
         p(i)=(1-2*Ff-deltat/T2f)*m(i)+2*Ff*m(i+1)
         write(6,*) i, p(i)
       else if (i .lt. IIDINT(a/deltar+1)) then
         p(i)=(Ff-Gf(i))*m(i-1)+(1-2*Ff-deltat/T2f)*m(i)
         +(Ff+Gf(i))*m(i+1)
         write(6,*) i, p(i)
       else if (i .gt. IIDINT(a/deltar+1) .and. i .lt. nr+1) then
         q(i)=(Fm-Gm(i))*n(i-1)+(1-2*Fm-deltat/T2m)*n(i)
         +(Fm+Gm(i))*n(i+1)
         write(6,*) i, q(i)
       else if (i .eq. nr+1) then
         q(i)=(1-2*Fm-deltat/T2m)*n(i)+2*Fm*n(i-1)
         write(6,*) i, q(i)
       endif
       
20      Continue
       p(IIDINT(a/deltar+1))=(Dm*q(IIDINT(a/deltar+1))+
                         K**(2/3)*Df*p(IIDINT(a/deltar+1)))/(K**(2/3)*Df+K*Dm)
       q(IIDINT(a/deltar+1))=K*p(IIDINT(a/deltar+1))
       write(6,*) IIDINT(a/deltar+1), p(IIDINT(a/deltar+1))
       write(6,*) IIDINT(a/deltar+1), q(IIDINT(a/deltar+1))
       
Use Trapezoidal Rule to add up magnetization

        da=IIDINT(a/deltar+1)
Appendix A. Source Code for $T_2$ Simulations

```fortran
sump=0
sump=deltar**2*(0*p(1)+nr*p(nr+1))
sumq=0
sumq=deltar**2*(0*q(1)+nr*q(nr+1))
Do 40 i=2,nr
    sump=sump+(i-l)*deltar**2*p(i)
    sumq=sumq+(i-l)*deltar**2*q(i)
40 Continue
sumtotal=sump+sumq

write(6,101)j,sump,sumq,sumtotal
write(Test,102)j,sump,sumq,sumtotal

c

c Save relevant decay points

tester=nt/JFIX(nt*deltat)*10
if (j/tester*tester .eq. j) then
    counter=counter+1
    decayp(counter)=sump
    decayq(counter)=sumq
    decayt(counter)=sumtotal
    write(6,*j,counter,decayp(counter))
    write(6,*j,counter,decayq(counter))
    write(6,*j,counter,decayt(counter))
    write(IData,101)counter*0.01,decayt(counter)
endif

Do 30 i=1,nr+1
    m(i)=p(i)
    n(i)=q(i)
30 continue

50 continue

close(unit=IData)
close(unit=Test)

101 Format(2E15.6)
102 Format(2X,12X,F13.10,2X,F13.10,2X,F13.10)
stop
end
```