The Use of
Quantitative Magnetic Resonance Imaging
in Perinatal Brain Injury

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ABSTRACT

The clinical signs and symptoms of hypoxic-ischaemic encephalopathy in human neonates are usually masked by the treatment administered to them. Hence a greater reliance on non-invasive monitoring techniques is required to aid prognosis. This thesis presents results of quantitative MRI techniques that are currently under development in order to study perinatally asphyxiated infants.

Quantitative ADC and T₂ measurements were made in a piglet model of perinatal hypoxic ischaemic brain injury. The global directionally averaged ADC (ADCav) falls during the 48 hour period of monitoring following the transient hypoxic-ischaemic insult. There is also a gradual rise in T₂ values during the 48 hour period. ³¹P MRS shows a decrease in [PCr]/[Pᵢ] ratio during this same period. An early significant (p < 0.05) drop in global ADCav occurs at four hours post hypoxia-ischaemia. Additionally, a parasagittal pattern of cerebral injury is discernible in ADCav maps. A strong relationship between changes in ADCav and [PCr]/[Pᵢ] is demonstrated (r = 0.95).

The quantitative relationship between R₂* and deoxyhaemoglobin was investigated using the piglet model. R₂* is found to correlate strongly with absolute deoxyhaemoglobin concentration ([dHb]) as measured by near infra red spectroscopy (r = 0.95). It is hoped that quantitation of R₂* can provide a non-invasive regional measure of cerebral deoxyhaemoglobin concentration in the perinatally asphyxiated infant. However, R₂' may provide a more sensitive measure of [dHb].

Hence an imaging sequence was developed allowing the acquisition of both T₂ and T₂* weighted images from the same NMR signal, enabling these parameters to be rapidly quantified in future studies. The pulse sequence utilises spin coherence generated by a stimulated echo experiment. The sequence makes it possible to investigate the line broadening component, T₂' of T₂*-weighted signal, as this component is believed to be a more specific measure of cerebral tissue changes.
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CHAPTER 1

Introduction
CHAPTER 1

Introduction

1.1 Hypoxic-Ischaemic brain injury in the neonate (Perinatal Asphyxia)

"Acute perinatal asphyxia refers to a condition of hypoxaemia, hypercapnia and insufficient blood perfusion of the newborn during labour and birth". "Hypoxic-ischaemic injury initiates disturbances of oxidative metabolism in the perinatal brain and is the most common cause of disabling brain injury in survivors of neonatal intensive care". The incidence of hypoxic-ischaemic encephalopathy in full term infants in the UK is reported to be between four and eight per 1000 live births. However, up to 10% of small preterm infants suffer ischaemic injury.

A healthy term neonate when subjected to an acute period of hypoxia responds with a series of effective physiological responses. Initially the heart rate falls, whilst the stroke volume of the heart increases. The peripheral vasculature undergoes vasoconstriction, increasing the arterial blood pressure and hence blood flow to essential organs such as the brain, heart and adrenals. Additionally, a rise in plasma catecholamines acts to increase the breakdown of glycogen stores, thereby elevating the blood glucose concentration. For approximately 10-15 minutes, whilst cardiac output is preserved through anaerobic metabolism of glucose and glycogen, glucose delivery to the brain is maintained at supranormal levels.

Under physiological conditions 1 mol of glucose produces 38 mol of Adenosine Triphosphate (ATP). ATP is the primary source of energy for cells. 36 of the 38 mol of ATP produced per mol glucose are formed by oxidative phosphorylation in mitochondria, only 2 mol ATP are produced from the glycolytic pathway. When oxygen delivery is curtailed oxidative phosphorylation is inhibited and glycolysis becomes the only method of ATP generation. However, the neonatal brain is extremely efficient at metabolising glucose and energy requirements can be met
through anaerobic glycolysis alone. If glucose delivery to the brain is maintained the neonatal brain can exist in this state for an indefinite period. However, because of an exhaustion of the cardiac glycogen stores, and a progressive worsening of acidosis, cardiac output falls, causing cerebral glucose delivery to be curtailed. This results in cerebral ischaemia.

Consequently, there is a gradual depletion of high energy phosphates, mainly ATP and Phosphocreatine (PCr) within brain cells. As a result a number of potentially cytotoxic biochemical processes are initiated that may lead to permanent brain injury even upon reperfusion. Namely, the development of acidosis, the accumulation of cytotoxic amino acids, the generation of oxygen derived free radicals and calcium ion intoxication.

The clinical signs of a newborn infant exposed to hypoxia-ischaemia are in some cases difficult to interpret. Therefore, a greater reliance on non-invasive methods of brain investigation is essential. A comprehensive assessment of perinatal asphyxia involves several sequential neurological examinations, cranial ultrasound and encephalographies in order to determine the prognosis at an early stage. Other techniques for aiding the accurate assessment of prognosis include near infrared spectroscopy (NIRS), cerebral blood flow measurements, evoked potentials, computed tomography (CT), Doppler recordings, Phosphorous ($^{31}$P) magnetic resonance spectroscopy (MRS) and magnetic resonance imaging (MRI).

Transient perinatal hypoxia-ischaemia leads to biphasic changes in cellular energy metabolism: a largely reversible impairment during the insult, and a secondary derangement beginning 9 to 24 hours later. In newborn infants that have suffered from perinatal asphyxia, the severity of delayed energy failure correlates with the degree of later neurodevelopmental impairment. The period before the delayed injury offers potential for therapeutic interventions, and subtle reductions in brain temperature after hypoxia-ischaemia may offer clinically valuable protection.

A model of perinatal brain injury following transient hypoxia-ischaemia has
been developed by our group in order to evaluate clinically feasible neuronal rescue strategies \(^{16,17}\). The aims of the project using the model are:

- To optimise the intensity and duration of mild hypothermia, commenced following reversal of a transient hypoxic-ischaemic episode, required to prevent or reduce delayed energy failure and ameliorate the histological evidence of permanent brain injury. The effect of magnesium therapy with and without hypothermia are also being investigated.

- To investigate the potential of MRI, MRS, NIRS and electrophysiological measurement applied during the critical period immediately following resuscitation after perinatal asphyxia to predict the development of delayed energy failure and ultimate extent of permanent brain injury. The intention is to develop non-invasive techniques whereby infants at risk of severe neurodevelopmental impairment or death can be selected for cerebroprotective therapy.

- Investigate the effect of increased brain temperature on the development of delayed energy failure in order to improve existing clinical guidelines for the management of asphyxiated infants.

This thesis describes our approach and the results from addressing the second of these aims. Metabolic measurements were performed using \(^{31}\)P and proton (\(^1\)H) MRS and quantification of the apparent diffusion coefficient (ADC) of tissue water and spin-spin relaxation time (T\(_2\)) map. The advantage of this approach over other imaging techniques is that all these measurements can be performed non invasively using non-ionising radiation in the human infant.

\section*{1.2 The Development of Nuclear Magnetic Resonance (NMR)}

The phenomena of NMR was first demonstrated in 1946 by two independent groups headed by Bloch and Purcell. Both reported observations in the same issue of
Physical Review 18, 19. Following NMR's initial demonstration, it was quickly established that the resonance frequency of $^1$H nuclei was dependent upon molecular environment. Due to the different electronic structures of various molecules, nuclei are screened from the applied magnetic field by differing amounts. Hence, they experience a local magnetic field that is different from the applied static field. The screening depends upon the compound in which the nucleus is situated and on the location of the nucleus within the molecule. Nuclei in chemically-distinct sites resonate at slightly different frequencies. Therefore, it is possible to identify the molecular environment of nuclei by the resonant frequency shift. To compare data from magnets of differing strengths the frequency shift is normalised and called the chemical shift. Chemical shifts were initially used to identify the relative amounts of particular molecules in solution. The first observations of chemical shifts were made by Proctor and Yu 20, and independently by Dickenson 21. With advances in magnet technology it became possible to look at the chemical shifts of $^{31}$P containing compounds in biological tissue, which had not been possible before because of the low relative sensitivity of $^{31}$P (relative sensitivity = $6.63 \times 10^{-2}$) as compared to the $^1$H (relative sensitivity = 1) nucleus. In 1973 Moon and Richards conducted studies of $^{31}$P in blood cells 22. More recently techniques have been developed by which MRS may be used to monitor the local concentrations of various metabolites in the human body in vivo 23.

The field of MRI was developed much later than MRS. In 1973 in a letter to Nature, Lauterbur was the first to publish an MRI image of a heterogeneous object comprising two tubes of water 24. At the same time Mansfield and Grannell 25 had developed the same method of spatial localisation which depended upon varying the resonant frequency of nuclei by application of a linear field gradient such that the resonant frequency of a nucleus is dependent upon position within the gradient.

The first human image of a live finger was produced by Mansfield and Maudsley in 1976 26. This was quickly followed in 1977 by the first image of a hand 27. Damadian was the first to produce a whole body image of a thorax in 1977 28. The following year Mansfield et al reported the first abdominal MRI scan 29. In the same
year, the first commercial MRI scanner became available. In 1980, Hawkes demonstrated the first images of abnormal human pathology.

The major $^1\text{H}$ containing molecule in the human body is water. The measurement of water content alone provides a contrast range of approximately 15% between soft tissues in the human body. Following these early images, the development of new pulse sequences means that it is now possible to obtain contrasts dependent upon a multitude of factors. Recent development has seen the emergence of contrast that is not just dependent upon the water content or structure of tissue, but also reflects tissue function. For example, it is possible to produce MRI images depicting microscopic motion (tissue water diffusion or blood perfusion), macroscopic motion (blood flow), local magnetic field susceptibility variations, and spatial distributions of biochemically important metabolites. However, single MRI images are weighted by a mixture of contrast mechanisms. As a result, clinical abnormalities in an image may appear hypointense, hyperintense or even isointense depending upon the mixture of relaxation weightings. This makes interpretation and comparison of images acquired at different magnetic field strengths difficult. Recent development in pulse sequence design has meant that it is possible to produce images that reflect only one particular contrast parameter. This is the process of quantitation.

For example a standard spin echo image is weighted by both spin-spin relaxation ($T_2$) and spin-lattice relaxation ($T_1$) (discussed further in chapter 2) contrast, with the exact weighting dependent upon pulse sequence timing. In a study using the spin echo technique it is crucially important to manipulate the sequence timing to give adequate discrimination of the anatomical features under investigation. Without prior knowledge of $T_2$ and $T_1$ for the sample, a single image may fail to provide the desired contrast. By quantitation of $T_2$ or $T_1$ it is possible to remove this uncertainty. Recent trends in MRI have been to quantitatively study contrast parameters such as ADC, $T_2$, $T_1$ and $T_2^*$ ($T_2^* = 1/T_2 + 1/T_2'$ where, $T_2'$ is the relaxation time for nuclei experiencing microscopic magnetic field inhomogeneity gradients). A series of images are usually taken, with the pulse sequence
varied so as to modify signal differences between images based on a single contrast mechanism.

1.3 Other Imaging Techniques

Current clinical practice utilises a number of imaging techniques. Each technique has advantages and disadvantages in diagnostic use. In this section the most common imaging techniques are discussed. These include X-ray CT, Ultrasound, Positron Emission Tomography (PET), MRI and NIRS.

Early medical imaging was exclusively based on X-ray examination of hard tissue. With the advent of CT, X-radiology quickly became important in imaging soft tissues. X-radiology relies upon the attenuation of X-rays by atoms. X-rays that aren’t attenuated reach a X-ray sensitive film causing the film to darken. Currently X-rays are still the method of choice for imaging hard tissue (bone, teeth etc.). In contrast, MRI signal from hard tissue decays before it can be acquired. MRI is now quickly becoming the modality of choice for many clinical examinations of soft tissue. Both techniques provide good spatial resolution (on the order of 1 mm) and can be used for anatomical investigation. In addition, MRI can also be used to study tissue function. MRI also benefits over X-rays from the versatility of image contrast. By simple manipulation of experimental parameters, it is possible to produce images weighted by various biophysical parameters. X-ray imaging on the other hand, has a smaller range of contrast parameters. Another benefit of MRI, lies in the use of non-ionising radiation, thereby limiting the radiation exposure hazard to the patient. However, both MRI and X-ray CT equipment require a substantial initial capital investment.

Non-ionising radiation is also used in ultrasound imaging. Sound energy is transmitted into the patient and an echo is received from boundaries between different tissues, thus producing an image. Ultrasound, like MRI, can provide anatomical and functional information. However, it has a more limited range of use within the body,
due to the attenuation of sound by deeper body structures. Additionally, ultrasound suffers from poor spatial resolution in comparison to X-rays and MRI. The major benefits of ultrasound are relative cheapness, and the ability to conduct a bedside scan, minimising patient risk.

PET provides a good method for studying tissue function. Positron emitting nuclei can be attached to specific metabolites and their distribution within the body imaged. PET produces images of a much poorer spatial resolution than MRI. Like MRI it can be used to study metabolites in vivo, however, PET uses ionising radiation which is hazardous to the patient.

Although NIRS is not an imaging technique, it provides invaluable in vivo measurements of deoxyhaemoglobin (dHb), oxyhaemoglobin (HbO₂) and cytochrome oxidase (CtOx) concentrations. Additionally it may be used to assess blood flow and blood volume (discussed further in Chapter 2). NIRS measures the transmission of near infra red light at specific wavelengths characteristic of individual chromophores (HbO₂, dHb and CtOx). Use of non-ionising radiation and the ability to be employed at the bedside make NIRS an important clinical monitoring technique. Current NIRS research is aimed at multi-optode scanners that allow not only spectroscopic measurements to be made, but can also produce images.

MRI, NIRS and ultrasound all use non-ionising radiations and, therefore, are suitable for neonatal investigations. Of these three, ultrasound and NIRS provide a cheap, portable form of neonatal examination. However, NIRS has no spatial resolution, and although ultrasound can produce images outlining anatomical information, the resolution of the technique is poor. In a MRI examination, although the neonate needs to be transported to the MRI scanner, images produced are of a high spatial resolution, providing accurate anatomical detail, and allowing regional abnormalities to be more easily identified. With regard to the ability of NIRS to measure absolute concentrations of its chromophores, preliminary studies using T₂*-weighted imaging have demonstrated the potential of MRI to provide regional information on dHb concentration.
These advantages offered by MRI makes MRI a useful tool for neonatal examinations.

1.4 Contents and Organisation of the Thesis

Chapter 2 of the thesis describes the theory of MRS, MRI and NIRS together with the relevant instrumentation. Chapter 3 concentrates on the physiological importance of MRS and MRI parameters that form the basis of the experiments carried out in the rest of the thesis.

Chapter 4 describes the development of quantitative MRI and MRS studies in a piglet model of perinatal asphyxia. The aim is to establish early markers for regional brain injury in perinatally asphyxiated human infants. It has previously been reported that a delayed secondary energy failure occurs after an initial acute hypoxic-ischaemic episode. The sequence of events that occur following the disruption of energy metabolism has been reproducibly demonstrated in the neonatal piglet model. The severity of the insult and subsequent variations in brain energy metabolism have been measured using \(^{31}\text{P}\) MRS. Chapter 4 presents the results of quantitative measurement of ADC and \(T_2\) in the neonatal piglet brain during the 48 hour time course following the ischaemic episode. The chapter further discusses physiological changes subsequently monitored using MRI and MRS. Additionally, the use of the ADC of cerebral tissue water as an early marker of cerebral injury is discussed. MRI provides high spatial resolution enabling the temporally varying regional distribution of cerebral damage to be assessed during secondary energy failure.

Chapter 5 describes experiments performed to quantitatively investigate the relationship between \(T_2^*\) and absolute deoxyhaemoglobin concentration ([dHb]) in the neonatal piglet brain. [dHb] is assessed by NIRS. This study demonstrates the dependence of absolute \(R_2^*\) values upon the concentration of cerebral dHb. Experiments were carried out using the newborn piglet model developed in our
laboratory, with a controlled step-wise oxygen (O₂) desaturation and concurrent quantitation of $R_2^*$ at each stable desaturation step.

$T_2^*$ weighted MRI has recently been used to investigate brain function, trabecular bone structure and brain iron $^{35,38,39}$. $T_2^*$ weighting involves both $T_2$ and $T_2'$. It is believed that $T_2'$, the line broadening component, is the more specific measure for these investigations. To determine $T_2'$, a quantitative approach is required with calculation of both $T_2$ and $T_2^*$ values. Chapter 6 describes the development of a new pulse sequence that allows the simultaneous quantitation of both $T_2$ and $T_2^*$ parameters using a hitherto unexploited form of stimulated / gradient echo.

Experimental phantom results using this sequence are presented and equivalently weighted images obtained with standard spin echo and Fast Low Angle Shot (FLASH) techniques are presented for comparison.

Finally Chapter 7 presents a summary of the results. In addition, an outline of future work building on the contents of this thesis is presented.
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CHAPTER 2

Nuclear Magnetic Resonance
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Nuclear Magnetic Resonance

2.1 Nuclear Spins in a Magnetic Field

An atomic nucleus with an odd mass number and/or an odd atomic number possesses a non-zero quantum spin number \( I \), associated with the intrinsic angular momentum of the nucleus. For an atom the total magnetic moment \( \mu \) is proportional to the combined angular momentum of the orbiting electrons and spinning nucleus within the atom, \( J \).

\[
\mu = \gamma J \tag{2-1}
\]

where \( \gamma \) is the proportionality constant called the gyromagnetic ratio. For the proton, \( \gamma = 2.675 \times 10^8 \text{ rad T}^{-1} \text{ s}^{-1} \) or 42.57 MHz T\(^{-1}\).

The angular momentum operator of a nucleus can be expressed by:

\[
\hat{J} = \hbar \hat{\mathbf{I}} \tag{2-2}
\]

and \( \hbar = h/2\pi \), where \( h \) is Planck’s constant \((6.626 \times 10^{-34} \text{ J s})\). \( \hat{\mathbf{I}} \) is the dimensionless spin operator with eigenvalues \([I(I+1)]^{1/2}\). \( \hat{I}_z \) has eigenvalues \( m \), with \( 2I+1 \) possible values of \( I, I-1, ..., -I \).

From equations [2-1] and [2-2], the magnetic moment of a nucleus has discrete values, and \( \mu_z \) has eigenvalues:

* Vectors are indicated in bold print. Operators are indicated by a chevron.
\[ \mu_z = \gamma m \quad m = 1, I - 1, ..., -I \]  \[2-3\]

In the presence of an external uniform magnetic field \((B_0)\), the interaction of the nucleus is given by the Hamiltonian or the energy operator:

\[ \hat{H} = -\hat{\mu} \cdot B_0 \]  \[2-4\]

For the case when \(B_0\) is orientated parallel to the \(z\)-axis:

\[ \hat{H} = \gamma \hbar B_0 \hat{I}_z \]  \[2-5\]

Therefore the allowed energies are given by:

\[ E = -\gamma \hbar B_0 \quad m = 1, I - 1, ..., -I \]  \[2-6\]

For the proton, which has a spin \(I = 1/2\), \(m\) can only have values of \(\pm 1/2\) and so there are only two nuclear energy states possible in the presence of \(B_0\). The orientation of \(\mu\) for the lower energy spin state is parallel alignment with \(B_0\), whereas the high energy state is anti-parallel. The energy difference between the two states is given by (fig [2-1]):

\[ \Delta E = \gamma \hbar B_0 \]  \[2-7\]

For transitions between the energy states to occur, energy must be either absorbed or emitted by the system, and this energy is given by:

\[ \Delta E = \hbar \omega \]  \[2-8\]

where \(\omega\) is the angular frequency.
For a bulk sample, the nuclei constantly change between the energy states. At thermal equilibrium the populations follow a Boltzmann distribution which is given by:

\[
\frac{N_-}{N_+} = \exp \left( - \frac{\Delta E}{kT_p} \right) \tag{2-9}
\]

where \( N_- \) and \( N_+ \) are the number of nuclei in the upper and lower energy states respectively, \( k \) is Boltzmann’s constant \((1.381 \times 10^{-23} \text{ J K}^{-1})\), and \( T_p \) is the temperature of the sample. The total number of nuclei is \( N = N_- + N_+ \) and the difference in the populations is given by equation [2-10]. At room temperature in a 7 Tesla (T) \( B_0 \) field the \( \tanh(x) \) term of equation [2-10] approximately equals \( x \), and for protons the population difference is approximately 24 spins per million nuclei.

\[
N_+ - N_- = N \tanh \left( \frac{\gamma \hbar B_0}{2kT_p} \right) \tag{2-10}
\]

and assuming \( \gamma \hbar B_0 \ll kT_p \):

\[
N_+ - N_- = \frac{N \gamma \hbar B_0}{2kT_p} \tag{2-11}
\]

At thermal equilibrium the net magnetisation \( M \), which is the sum of the precessing magnetic moment vectors of all the nuclei is given by:

\[
M = N_+ \mu_+ + N_- \mu_- \tag{2-12}
\]


\[
M = \frac{\gamma \hbar}{2} (N_+ - N_-) = \frac{N \gamma^2 \hbar^2 B_0}{4kT_p} \tag{2-13}
\]
Since the population difference and the net magnetisation are small, NMR is not a very sensitive technique. However, the $^1$H nucleus has almost 100% natural abundance, has the highest intrinsic sensitivity (1.0), and is the commonest nucleus in biological systems. Signals from metabolites containing $^1$H nuclei are, therefore, readily detectable.

The net magnetisation vector $\mathbf{M}$ for a system of non-interacting spins obeys the classical equation of motion for a magnetic moment experiencing a torque due to an applied external magnetic field $\mathbf{B}$ (see equation [2-14]).

$$\frac{d\mathbf{M}}{dt} = \gamma \mathbf{M} \times \mathbf{B} \quad [2-14]$$

Equating equations [2-7] and [2-8] gives equation [2-15] for the rate of precession of the magnetic moment vector about $\mathbf{B}_0$, where $\omega_0$ is the Larmor frequency of precession.

$$\omega_0 = \gamma B_0 \quad [2-15]$$

A radiofrequency (RF) pulse at an angular frequency $\omega_0$ generates a linearly polarised alternating magnetic field. This field can be represented by two counter-rotating components, each of strength $B_1$. One component rotates in the same sense as the magnetic moment precession and the other in the opposite sense. The total applied external field is given by:

$$\mathbf{B}_{\text{tot}} = \mathbf{B}_0 + \mathbf{B}_1 \quad [2-16]$$

$\mathbf{M}$ experiences a torque due to $\mathbf{B}_{\text{tot}}$, and so simultaneously precesses about $\mathbf{B}_0$ and $\mathbf{B}_1$. In the laboratory frame this is a spiral motion (fig [2-2]).
2.2 Rotating Frame of Reference

To understand the concepts of NMR easily, it is more convenient to deal with a rotating frame of reference rather than static laboratory frame. The axes x', y' and z' rotate about $B_0$ so that z and z' are parallel, and the x' axis is along the $B_1$ direction. The effective field $B_{\text{eff}}$ is given by:

$$B_{\text{eff}} = B_0 + \frac{\omega}{\gamma} + B_1$$  \hspace{1cm} [2-17]

The magnetisation then responds to this effective field according to the equation:

$$\frac{dM}{dt} = \gamma M \times B_{\text{eff}}$$  \hspace{1cm} [2-18]

In the rotating frame, M experiences a torque due to the effective magnetic field $B_{\text{eff}}$ and so precesses about $B_{\text{eff}}$ at an angular frequency $\gamma B_{\text{eff}}$ (fig [2-3]).

At resonance i.e. $\omega = \omega_0$ and $B_{\text{eff}} = B_1$. M experiences a torque due to $B_1$ only, and so precesses about $B_1$. A RF pulse of duration $\tau$ rotates M about the x' axis through an angle $\theta$ (measured in radians) given by:

$$\theta = \frac{\gamma B_1 \tau}{\gamma}$$  \hspace{1cm} [2-19]

For a 90° pulse, M simply flips from the z' axis to the y' axis. At the end of the pulse, M precesses at the frequency $\omega_0$ in the laboratory frame, but is static in the rotating frame.
2.3 Relaxation

Once $\mathbf{M}$ has flipped into the $xy$ plane and $\mathbf{B}_f$ is removed, $\mathbf{M}$ relaxes back to equilibrium via two different mechanisms - the $T_1$ and the $T_2$ relaxations. For efficient relaxation, fluctuating magnetic field components are required at $\omega_0$. There are a number of mechanisms by which these fluctuations are produced. One such mechanism is the interaction between magnetic dipoles. A given nucleus experiences a magnetic field generated by it's neighbours, which is dependent upon their geometrical arrangement and spin dispositions. If that nucleus moves relative to it's neighbours or if the neighbours move, there is a change in the local field.

The spin-lattice or longitudinal relaxation involves the spin states losing energy to the surrounding molecular environment. The $z$ component of the magnetisation vector ($M_z$) returns to its equilibrium value $M$. The relaxation is exponential with decay time $T_1$. After approximately $5T_1$'s -99% relaxation has occurred. It is the components of molecular motion close to the $\omega_0$ that produces magnetic field fluctuations which are effective in inducing $T_1$ relaxation.

$T_2$ or transverse relaxation involves the exchange of energy between the spin states with the result that any transverse magnetisation ($M_{xy}$) decays to zero. The relaxation is assumed to be exponential with decay time $T_2$. Similar mechanisms to those responsible for spin lattice relaxation are involved. Additionally, there is a low frequency component whereby relaxation occurs because neighbouring nuclei experience slight variations in the local $B_0$ field. Therefore, nuclear magnetic moments precess at different frequencies in the $xy$ plane, lose phase coherence, and the net magnetisation $M_{xy}$ signal is decreased. This component to $T_2$ relaxation can be explained using the Hisenberg uncertainty principle:

$$\Delta E \cdot \Delta t \geq \frac{h}{2\pi}$$  \[2-20\]
where $\Delta E$ is the uncertainty in energy, $\Delta t$ the uncertainty in time, and $\hbar$ is Planck’s constant. In a sample of nuclei, if the uncertainty in energy, $\Delta E$, is large, then $\Delta t$ is small, i.e. $T_2$ is short.

In the rotating frame, if $\mathbf{B}_1$ is aligned along the $x'$ axis, the motion and relaxation of $\mathbf{M}$ is described by the modified Bloch equations:

\[
\frac{dM_z}{dt} = -\gamma B_1 v \left( \frac{M_z - M_0}{T_1} \right) \tag{2-21}
\]

\[
\frac{du}{dt} = +\left( \omega_0 - \omega \right)v \frac{u}{T_2} \tag{2-22}
\]

\[
\frac{dv}{dt} = -\left( \omega_0 - \omega \right)u + \gamma B_1 M_z \frac{v}{T_2} \tag{2-23}
\]

where $u$ and $v$ are components of magnetisation perpendicular to $B_0$, $u$ is the component in the direction of $B_1$ and $v$ is the out of phase component along the $y'$ axis such that:

\[
u = M_x \cos \omega t - M_y \sin \omega t \tag{2-24}
\]

\[
v = M_x \sin \omega t + M_y \cos \omega t \tag{2-25}
\]

In addition to these signal decay processes, the presence of magnetic field inhomogeneities may also act to dephase spins around the precessionary orbit. This results in dephasing in the $xy$ plane and is called $T_2'$ relaxation. The combined effect of $T_2$ and $T_2'$ relaxation is referred to as $T_2^*$ relaxation where $T_2^*$ is defined as:

\[
\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'} \tag{2-26}
\]

$T_2$ relaxation is irreversible. However, dephasing caused by $T_2'$ can be reversed by using a “refocussing” pulse to produce a spin-echo.
2.4 **The Spin-Echo**

The spin echo pulse sequence is shown in fig [2-5] and is represented in shorthand notation as:

\[ 90^\circ \rightarrow \text{TE/2} \rightarrow 180^\circ \rightarrow \text{TE/2} \rightarrow \text{acquire} \rightarrow \text{TR} \]

where TE is the echo time.

A 90° RF pulse rotates the magnetisation vector \( \mathbf{M} \) about \( x' \) to the \( y' \) axis (fig [2-4]). \( T_2^* \) relaxation occurs during the period \( \text{TE/2} \) resulting in a dephasing of individual magnetisation vectors from the \( y' \) axis. After the period \( \text{TE/2} \) a "refocussing" pulse is applied along the \( x' \) axis which rotates the magnetisation vectors by 180°. The individual magnetisation vectors continue to precess at their original frequencies and refocuss along the \( -y' \) axis at a time \( \text{TE} \) after the 90° RF pulse (fig [2-4]). The spin echo sequence is able to reverse the dephasing process represented by \( T_2^* \) but \( T_2 \) relaxation is unaffected. As a function of echo time the signal is given by:

\[ S(\text{TE}) = A e^{-\frac{\text{TE}}{T_2}} \quad [2-27] \]

where \( A \) is the amplitude of the signal immediately after the 90° pulse.

2.5 **NMR Signal (Free Induction Decay)**

After the 90° pulse, \( M_{xy} \) is detected by the oscillating voltage magnetically induced in a RF receiver coil. The voltage, which oscillates at a frequency \( \omega_0 \) and decays exponentially with time \( t \), is called the free induction decay (FID) signal.
Any function can be represented as a summation of sine and cosine functions and, therefore, has an associated frequency spectrum. The FID signal is usually processed by Fourier Transformation (FT). This is a mathematical technique that analyses a signal from the time domain $S(t)$ to obtain the frequency spectrum, $Y(\omega)$, and is defined by the equation:

$$Y(\omega) = \int_{-\infty}^{\infty} S(t) e^{-i\omega t} dt \quad [2-28]$$

### 2.6 Spatial Encoding and Imaging

Magnetic Resonance Imaging techniques determine the spatial localisation of NMR sensitive nuclei. Localisation along the x, y and z directions is accomplished by three separate processes, namely frequency encoding, slice selection and phase encoding.

#### 2.6.1 Frequency Encoding

Following a 90° pulse, nuclei precess about $B_0$ at the Larmor frequency $\omega_0$. Ignoring field inhomogeneity effects, all spins resonate at the same frequency. A magnetic field gradient applied along the x direction causes neighbouring spins within the gradient to precess at different frequencies. Frequency encoding involves acquisition of an echo in the presence of a linear magnetic field gradient. Frequency analysis of the echo by Fourier transformation produces a profile of the sample.

#### 2.6.2 Slice Selection

Protons in the imaging slice are selectively excited by a combination of frequency-selective RF pulse and a field gradient applied perpendicular to the imaging plane. The bandwidth of the RF pulse and the amplitude of the slice-selection gradient determine the slice thickness. Slice thickness can be reduced by increasing the gradient.
strength or by decreasing the RF bandwidth. During the application of the slice selection gradient, spins across the thickness of the slice acquire a relative phase angle as they are being excited. Therefore, a second refocussing gradient of opposite sign to the original slice selection gradient, is applied causing the spins to rephase.

2.6.3 Phase Encoding

To obtain a second axis of spatial information spins are subjected to a magnetic field gradient ($G_y$), perpendicular to both the frequency and slice selective gradients, for a period $\Delta t_y$ (fig [2-5], period 2). During this period, spins located along the y axis precess at different rates and, hence, accumulate different phase angles $\phi$ by the end of the phase encoding period $\Delta t_y$. At this time the gradient $G_y$ is turned off and spins retain a knowledge of their evolution history through their so called phase memory. An echo is then acquired in the frequency encoding axis, and the pulse sequence repeated for incremented phase encoding gradient amplitudes. Increasing the phase encoding gradient in subsequent experiments results in spins having accumulated more phase angle by the end of $\Delta t_y$, and each new line is placed in a 2D array creating a second axis of signal evolution behavior. Therefore, from a series of experiments it is possible to analyse the evolution of phase angle along this 2nd axis in an analogous manner to the frequency encoding axis. Two dimensional Fourier transformation is then used to determine the position of each spin in the xy plane.

2.7 K-Space

A pulse sequence diagram (fig [2-5]) shows the gradients and pulses that need to be applied in order to sample data to fill a matrix of $N_x$ points (number of sample points for a single echo) by $N_y$ points (number of echoes sampled). A two dimensional Fourier transform of this time domain data provides the NMR image. In a similar manner to a pulse sequence diagram, the instructions necessary to fill up the data matrix can also be expressed in terms of K-space, (fig [2-6]). A wave can be represented by the equation:
\[ A(x,t) = A_0 e^{i(K_x-x)t} \]  \[2-29\]

where \( K = 2\pi/\lambda \). The NMR signal from spins at position \( x \) in a fixed gradient \( G_x \) may be represented as either:

\[
M(t) = M_0 e^{i\omega t} \]  \[2-30\]

or as \[
M(t) = M_0 e^{ikx} \]  \[2-31\]

where \( \omega = \gamma G_x \), and \( K = \gamma G_x t \) where \( t \) is time. If \( G_x \) varies with time, then \( K = \int \gamma G_x dt \). If the signal is sampled for a time \( t_{\text{max}} \), then:

\[
M(t_{\text{max}}) = M_0 e^{ik_{\text{max}}x} \]  \[2-32\]

where \( k_{\text{max}} \) is given by:

\[
k_{\text{max}} = \int_{0}^{t_{\text{max}}} \gamma G_x dt = \frac{2\pi}{\lambda_{\text{min}}} \]  \[2-33\]

\( \lambda_{\text{min}} \) represents the image resolution. From equation [2-33] it can be seen that high spatial frequencies are represented at the extremities of K-space and define high frequency information of the object being imaged. Low spatial frequencies represent the bulk structure of the object and are sampled in the centre of K-space.

The resolution of an image is limited by the highest spatial frequency that can be sampled (equation [2-33]). To increase the highest spatial frequency it is necessary to either increase the gradient strength (doubling the gradient strength doubles the resolution for a constant sampling interval) or to sample the signal for longer (equation [2-33]).

The pulse sequence diagram (fig [2-5]) for a spin echo sequence has a K-space representation as in fig [2-6]. During period 1 (fig [2-5]) the excitation pulse is applied
to create the transverse magnetisation; this corresponds to commencement of signal
evolution at the origin of K-space (fig [2-6]). During period 2 the phase encoding
gradient G_y and the frequency encoding gradient G_x are applied simultaneously (fig [2-
5]), which is represented in K-space by moving from A ⇒ B (fig [2-6]). In period 3 of
the pulse sequence a 180° pulse is applied (fig [2-5]) which is represented in K-space as
moving from B ⇒ C (fig [2-6]). Finally in period 4, a frequency encoding gradient is
applied (fig [2-5]) while the echo is read out represented as a movement from C ⇒ D
(fig [2-6]). The process is then repeated with a decremented phase encoding gradient,
thereby filling a different line of the time domain data matrix during signal acquisition.

Once the data matrix has been filled, a 2D FT is applied in order to produce the
final image.

2.8 The Extended Phase Graph Algorithm

The generation of an echo using a standard spin echo sequence can be easily
explained using a two vector model, with individual vectors representing nuclei that
experience a slightly different B_0 magnetic field. However, the formation of an echo as
a result of multiple RF pulses can no longer be explained simply using this conventional
vector model. For example, if a two vector model is applied to a stimulated echo pulse
sequence (consisting of three 90° RF pulses), it is not immediately apparent that an echo
will ever be generated, as the two vectors do not necessarily align along an individual
axis at the echo time. Using an eight vector model the situation is somewhat improved.
The formation of a stimulated echo is more easily visualised at a time TE, as all eight
vectors are brought into one half of the xy plane. The sum of these vectors generates a
non zero component along the x axis (the stimulated echo).

For magnetic resonance pulse sequences containing a greater number of RF
pulses, or pulses with low flip angles, a relatively large number of vectors are required if
the vector model is used. In such instances the conventional vector simulation becomes
cumbersome, and the extended phase graph algorithm (Hennig 1991) provides an easier
approach to understanding the formation of echoes.

This algorithm considers the total magnetisation to be a superposition of many
isochromats defined as: $F_n$, $F_n^*$, $Z_n$ and $Z_n^*$, where $n$ is an integer greater than zero.
These states are described pictorially in fig [2-7]. Each state represents a condition
under which all the transverse magnetisation vectors before the application of a RF pulse
are totally dephased.

The $F_1$ state is described as a complete dephasing of vectors after application of
a gradient pulse. The population of the $F_1$ state is set to 1 following a 90° RF pulse, or
sin $\alpha$ following an RF pulse with an arbitrary flip angle $\alpha$. A second configuration, $F_1^*$,
is described as that state created from $F_1$ by an inversion of the $y$ component of each
vector. The $z$ magnetisation is described as a combination of two counter rotating $z$
terms $Z_1$ and $Z_1^*$. The states $F_2$ and $F_2^*$, and, $Z_2$ and $Z_2^*$ represent vectors that have
dephased after the application of two gradient pulses of the same magnitude, duration
and sign.

Although the transverse magnetisation, given by the sum of all the vectors in any
state is zero, all the transverse magnetisation at the time of the application of an RF
pulse can be attributed to be in one or more of the states described by fig [2-7]. Using
this convention it is possible to keep track of all configurations of spins. This may be
plotted as an extended phase graph, as illustrated in fig [2-8]. Fig [2-8] shows a general
phase graph for a multiple pulse, multiple echo sequences with echoes being generated
when the $u,v$ line is crossed by a solid line.

The stimulated echo pulse sequence can be described easily using the extended
phase graph algorithm. Following an initial 90° RF pulse, after an evolution time $TE/2$,
spin vectors may be represented completely by the $F_1$ state, whose population is set to 1.
A second 90° RF pulse applied at this time acts to store some magnetisation in the $Z_1$
and $Z_1^*$ states, for the period of the mixing time $TM$. During $TM$ any remaining
transverse magnetisation in either the $F_1$ or $F_1^*$ states is scrambled by crusher gradients.
However, the $Z_i$ and $Z_i^*$ states are unaffected. A third 90° RF pulse transfers the population of the $Z_i$ state to the $F_i^*$ state. Finally, a further time $TE/2$ after the application of the third RF pulse, a stimulated echo is generated from the $F_i^*$ state.

### 2.9 NMR Spectroscopy

The ability to distinguish between various metabolites by spectroscopy arises from the difference in the local magnetic field experienced by nuclei in different molecules or located in various chemical groups in the same molecule. The local molecular electronic structure produces a local magnetic field at the nucleus that opposes $B_0$. The electrons shield or screen the nucleus from $B_0$, giving rise to an effective field $B_{0\text{(eff)}}$:

$$B_{0\text{(eff)}} = B_0 (1 - \sigma) \tag{2-34}$$

where $\sigma$ is the screening factor and depends on the local molecular environment of the nucleus. Therefore, nuclei resonate at slightly different frequencies depending on their local molecular environment. The main use of spectroscopy is for chemical analysis and molecular structure determination.

The difference in radiofrequency compared to a standard resonance is called the chemical shift and is quoted in parts per million (ppm) of the static magnetic field strength. The same nuclei observed at different field strengths will have the same chemical shift relative to a standard resonance. Chemical shift ($\delta$) is defined by the equation:

$$\delta = \left( \frac{\omega - \omega_{\text{ref}}}{\omega_{\text{ref}}} \right) \times 10^{-6} \tag{2-35}$$

where $\omega_{\text{ref}}$ is the frequency of a nucleus of a reference substance and $\omega$ is the resonant frequency of the nucleus of the substance of interest.
trimethylsilylpropionate (TSP) is used for proton studies because its chemical shift is very stable in different chemical and physical environments.

The chemical shifts of some resonances depend strongly on pH, temperature or metal ion concentrations. Hydrogen-bound (acid) and unprotonated (base) chemicals have different chemical shifts due to the difference in electronic structure. These can be rapid exchange between the acid and base states so a single resonance with the average chemical shift of the relative equilibrium populations is observed. The local concentration of hydrogen ions (and hence pH) determines the acid-base populations and so can alter the chemical shift. In a similar way metal-ions affect chemical shifts. Temperature affects the chemical bonds of molecules, the electronic structure, and hence the magnitude of chemical shift.

2.10 Near Infra-Red Spectroscopy

2.10.1 Basic NIRS Theory

Light in the near infra-red region of the spectrum (650-1000 nm) can be used to monitor concentration changes of dHb, HbO2 and CtOx in tissue. Light passing through tissue is attenuated by absorption and scattering. The quantity and wavelength of light depend upon the concentrations of the absorbing chromophores. For melanin, bilirubin, bone etc., this concentration is fixed over the measurement time. However, since concentrations of dHb, HbO2 and CtOx vary with oxygenation status in soft tissue, the absorption of near infra-red light at the characteristic wavelengths of these compounds also varies with oxygenation. The attenuation of light in tissue can be calculated using a modified Beer-Lambert law:

\[ A = \lg \frac{I_0}{I} = \alpha \cdot c \cdot l \cdot B + G \]  

where A is the attenuation measured in units of optical density (OD), I0 is the intensity of light incident on the tissue, I is the light intensity transmitted through the
medium, $\alpha$ is the specific extinction coefficient of the absorbing compound (OD $\text{molar}^{-1}\text{cm}^{-1}$), $c$ is the concentration of the absorbing compound in the tissue (molar), and $d$ is the distance between the points where the light enters and leaves the object (cm). To account for scattering the terms $B$ and $G$ have been included in the above equation. $B$ is the differential pathlength factor, which is a scaling factor that accounts for the extra distance travelled by the light in the tissue due to scattering effects. $G$ is a simple additive factor to account for scattering losses.

$G$ is unknown as this is dependent upon the exact geometry and the scattering coefficient of the tissue. Hence, absolute concentration of chromophores cannot be determined by simple application of equation [2-36]. However, if a change in attenuation is measured, then provided that $G$, $d$ and $B$ remain constant, a change in concentration can be calculated using the equation:

$$\Delta A = \Delta c\cdot \alpha\cdot d\cdot B \quad [2-37]$$

Fig [2-9] shows a schematic of the experimental set-up for NIRS measurements performed on the piglet head. Optical fibres carry near infra red light to the cylindrical optodes which direct the light normally onto the surface of the tissue. The inter optode spacing (IOS) is measured along a straight line between the two optodes using callipers.

2.10.2 Measurement of Absolute dHb/HbO$_2$ concentration

Equation [2-37] contains two unknowns; the concentration of the chromophore ($c$) and the differential pathlength factor ($B$). With knowledge of $c$, calculation of $B$ becomes possible. In the case of water, the water content of cerebral tissue has been measured in neonates using NMR techniques, and is believed to be in the region of 85%. Considering the attenuation of light as a consequence of tissue water, it is found that the attenuation is dependent upon both the concentration of water (absorption) and the differential pathlength factor (scattering). Measuring the attenuation of light in tissue and comparing the second differential of the spectral features of water at 710 and 830 nm with those of pure water, it is possible to calculate the differential pathlength since...
the concentration of water and the attenuation (absorption and scattering) are all known. The differential pathlength of light is independent of the absorbing chromophore, but dependent upon the scattering properties of the tissue. Hence, if the value for differential pathlength is substituted into equation [2-37], the attenuation measured for any chromophore at the same wavelength becomes proportional to the concentration of the chromophore and is independent of scattering. The technique of absolute quantification of deoxyhaemoglobin concentration involves measuring an attenuation spectrum for dHb between 650 and 1000 nm at 2 nm intervals using a charge coupled device (CCD) camera. However, quantitation from this spectrum is difficult because it has an arbitrary baseline value and broad spectral features. Using the double differential of the spectrum sharpens the features, and also gives a zero baseline. This allows calculation of absolute [dHb] using the equation:

$$[\text{dHb}]_A (\text{mM}) = \frac{[\text{dHb}]_m \times [\text{H}_2\text{O}]_A}{[\text{H}_2\text{O}]_m}$$  \hspace{1cm} [2-38]$$

where [dHb]A and [H2O]A (taken to be 85%) are the absolute concentrations of the respective chromophores and [dHb]m (mM⁻¹cm⁻¹nm⁻²) and [H2O]m (cm⁻¹nm⁻²) are the measured attenuations.

Absolute concentrations of HbO2 are determined by back-calculation from zero [HbO2]. Normally, absolute changes in relative concentration can only be measured (equation [2-37]) as there is no standard to which the near infra red light attenuation spectrum can be calibrated. For absolute quantitation the attenuation at the characteristic wavelength of HbO2 is measured upon death of the animal where [HbO2] is assumed to be zero. Having established a zero point, all changes of [HbO2] from zero can be determined absolutely.

2.10.3 Measurement of Absolute Cerebral Blood Volume (CBV)

Measurement of CBV involves making a small slow change to arterial oxygen saturation (SaO2). It is assumed that this change is sufficiently small to avoid changes in
cerebral blood flow (CBF), CBV and oxygen consumption. The change in HbO\textsubscript{2} is then equal to the total cerebral haemoglobin concentration tc[Hb] multiplied by the fractional change in SaO\textsubscript{2}.

\[ \Delta \text{HbO}_2 = tc[Hb] \cdot \Delta \text{SaO}_2 \] \hspace{1cm} [2-39]

If CBV is constant, then changes in HbO\textsubscript{2} and dHb will be equal and opposite and:

\[ \Delta \text{dHb} = -tc[Hb] \cdot \Delta \text{SaO}_2 \] \hspace{1cm} [2-40]

Therefore combining [2-39] and [2-40] we obtain:

\[ tc[Hb](\mu\text{molar}) = \frac{\Delta \text{HbO}_2 - \Delta \text{dHb}}{2 \cdot \Delta \text{SaO}_2} = \frac{\Delta \text{Hb}_{\text{diff}}}{2 \cdot \Delta \text{SaO}_2} \] \hspace{1cm} [2-41]

where \( \Delta \text{Hb}_{\text{diff}} = \Delta \text{HbO}_2 - \Delta \text{dHb} \).

For calculation of CBV in ml of blood per 100g of brain tissue, a constant X was used to take into account the molecular weight of dHb and tissue density; for the cerebral to large vessel haematocrit ratio (CLVHR) a value of 0.69 was used. Hence:

\[ \text{CBV}(\text{ml.100g}^{-1}) = \frac{X \cdot \Delta \text{Hb}_{\text{diff}}}{2 \cdot [\text{tHb} \cdot 10^{-2}] \cdot \Delta \text{SaO}_2 \cdot \text{CLVHR}} \] \hspace{1cm} [2-42]

where [tHb] is the concentration of haemoglobin in whole blood in g.100ml\textsuperscript{-1}.

To calculate CBV a change in Hb\textsubscript{diff} and SaO\textsubscript{2} are measured over the same period. A plot of \( \Delta \text{Hb}_{\text{diff}} \) verses SaO\textsubscript{2} is obtained and the slope of the regression line is used for the calculation of CBV in equation [2-41].
2.11 NMR Hardware

The Biospec (Bruker, Karlsruhe, Germany) 70 / 20 and 24 / 40 spectrometers utilise superconducting magnet systems and can perform both imaging and spectroscopy. Superconducting magnets are the most stable source for $B_0$. It is the only type of magnet that can achieve homogeneous fields greater than 1 T over the large volumes essential for in vivo spectroscopy. The 70 / 20 and 24 / 40 Biospecs operate at 7.0 T and 2.4 T field strengths respectively (giving resonant frequencies of 300.3 MHz and 100.3 MHz for $^1$H respectively), and have horizontal clear bores of diameters 21 cm and 40 cm respectively.

Inside the magnet are mounted the shim coils, the linear gradient field coils and the RF transmitter and receiver coil. Computer controlled variable current power supplies drive the shim and gradient coils. A block diagram of the spectrometer is shown in fig [2-10].

To obtain high resolution spectra, the magnetic field must be uniform to better than 1 part in $10^7$. The $B_0$ field is produced by a superconducting solenoid. It is designed to produce a uniform field over as large a volume as possible. The 2.4 T and 7 T systems should have a homogeneous volume of 19 cm and 8 cm diameter respectively. Since the mechanical layout of the solenoid or the solenoid position in the cryostat will never be exactly as designed, it is impossible to produce a perfectly homogenous magnetic field. Also, when a sample is placed in the magnet, the magnetic susceptibility of the sample often distorts the field. Shim coils improve the homogeneity. These are sets of superconducting and resistive coils wound so that they produce fields, generally of the order of a few mT, over the sample volume. The currents through the shim coils are adjusted so as to cancel out the inherent gradients. Since complex shapes like the human body produce complex field distortions, there is a high resolution shim system consisting of 15 shim coils.

Gradient coils produce fields that vary linearly across the sample. They are required for spatial localisation in imaging and localised spectroscopy. Three
orthogonal gradients, \( G_x, G_y \) and \( G_z \), are used with the origin defined as the magnet centre. The gradients are switched on and off very rapidly, typically 0.5 ms. This induces eddy currents in the magnet which cause time dependent \( B_0 \) perturbations that can be reduced with actively-shielded gradient coils in which the external field produced by the gradient coil is reduced so as to minimise the coupling with the main magnet. Both the 2.4 T and 7 T magnets have these gradient coils. Alternatively, `pre-emphasis' changes the shape of the gradient pulses so as to compensate for the eddy current effects.

A frequency generator (source) produces a continuous RF wave. According to the pulse program parameters, RF switching produces the pulse, which is then amplified and finally sent to the transmitter coil. The same coil can transmit the RF pulses to the sample and receive the resulting NMR signal. Alternatively, two coils can be used, a large transmitter and a smaller receiver.

For most of the studies at 7 T a surface (transmitter and receiver) coil was used. This design is often used because it achieves high signal to noise ratio (SNR), good localisation and has low power requirements. However it produces a highly inhomogeneous \( B_1 \) field. A single-turn surface coil of diameter 2.5 cm was used, with the impedance balanced-matched (inductively coupled) to the pre-amplifier output/input impedance of 50 \( \Omega \).

The FID signal picked up by the receiver coil is amplified in a low noise, fast recovery preamplifier. Phase sensitive quadrature detection is then applied which involves mixing the FID with a sine and cosine wave to produce real and imaginary components. The main receiver further amplifies the signal and changes the frequency from radio to audio frequencies for digitisation.

The Aspect 3000 is the central computer (24 bit) for the Biospec. Virtually all NMR pulse sequences can be generated and / or programmed. Pulse programs with a high degree of timing accuracy can be produced. Most of the spectrometer adjustments and the entire data acquisition process are under computer control.
Analogue-to-digital converters convert the receiver output (volts) to digits for input to the computer. The analogue to digital converters are of dual channel construction for quadrature detection, with dwell times of 4 μs to 4096 s in 1 μs steps and a resolution of 16 bits, both are under software control. The analogue to digital converter defines the dynamic range of the system i.e. a n-bit analogue to digital converter has a dynamic range of $2^{n-1}$:1.

The echo is discretely sampled for a finite time. N data points are sampled at interval $\Delta t$. It is sampled at the Nyquist frequency, i.e. at a rate of twice the highest frequency to be resolved, thereby preventing the aliasing of frequencies within the desired frequency range.

User commands are displayed on a visual display unit (VDU). A second VDU displays the FIDs, spectra and images and the parameters pertaining to the pulse sequence. Data is transferred from the spectrometer via ethernet to a Sun-Sparc (version 4) workstation where off-line data analysis is performed.
2.12 References

\[ \Delta E = \pm \frac{\gamma \hbar B_0}{2} \]

Fig [2-1] The nuclear spin energy for a single nucleus with \( I = 1/2 \) (such as \(^1\text{H}\)), plotted as a function of the magnetic field strength \( B_0 \). An allowed transition involves \( \Delta m = \pm 1 \).

Fig [2-2] The effect of a circularly polarised RF field on \( \mathbf{M} \), applied in a plane perpendicular to \( B_0 \), such that the magnetic component \( B_z \) lies in the \( xy \) plane.
Fig [2-3] Effective fields and the initial precession of the nuclear magnetisation vector $M_q$, in a rotating frame. The RF is applied with $\omega_1$ slightly below resonance.

Fig [2-4] A 90° RF pulse oriented along the x' axis in the rotating frame produces a net transverse magnetisation, $M_{xy}$. The $M_{xy}$ component of magnetisation dephases due to $B_0$ inhomogeneity. A 180° RF pulse oriented along x' rotates the y' component of magnetisation through 180°, causing spins to rephase at a later time.
Fig [2-5] In a spin echo sequence, a 90° RF pulse rotates \( M_\text{s} \) into the xy plane during period 1. Frequency and phase encoding gradients are applied during period 2. Magnetisation receives a 180° RF pulse during period 3 to produce an echo at time TE during period 4. The echo is sampled in a frequency encoding gradient.
Fig [2-6] K-space representation of a spin echo sequence. A 90° RF pulse initiates signal evolution at the origin of K-space. Positive frequency and phase encoding gradients applied during period 2 are indicated by a diagonal movement through K-space. A 180° RF pulse applied during period 3 causes movement from B to C. Finally, a positive frequency encoding gradient is applied and the signal is sampled. This is represented by a movement from C to D. The parital echo peak occurs as the signal is rephased at $K_y = 0$.

Fig [2-7] The configurations $F_i$ and $Z_i + Z_i^*$. The arrows indicate the sense of rotation with increasing frequency offset to the rotating reference frame. The $Z_i$ and $Z_i^*$ configuration always appear as a pair representing the linearly polarised configuration of z magnetisation.
**Fig [2-8]** The extended phase graph algorithm for a multiple pulse, multiple echo sequence. RF pulses are represented by two closely spaced vertical lines. Solid lines represent the time evolution of transverse magnetisation between pulses; the dotted lines represent configurations of $z$ magnetisation (Hennig 1991).

**Fig [2-9]** Schematic of the experimental setup for NIRS measurements across the head. In practice optodes are rarely positioned opposite each other. IOS = inter optode spacing.
Fig [2-10] Block diagram of the major components of an NMR imaging and spectroscopy system.
CHAPTER 3

Physiological Significance of NMR Measurements
CHAPTER 3

Physiological Significance of NMR Measurements

3.1 \( T_2^* \) Fast Low Angle SHot (FLASH)

3.1.1 Physiological Significance

Magnetic field inhomogeneities in vivo, are caused by the intrinsic variability in the magnetic properties of tissues. The strength of the applied external magnetic field is altered within a given tissue, by a factor \( M \), dependent upon the magnetic susceptibility (\( \chi \)) of the tissue.

\[
M = \chi \cdot B_0 \tag{3-1}
\]

where \( M \) is the induced magnetisation, \( \chi \) the susceptibility of the tissue and \( B_0 \) is the external magnetic field strength. In tissues where \( \chi \) is positive, then \( M \) acts to enhance the external magnetic field \( B_0 \); and for a negative \( \chi \), \( M \) acts to oppose \( B_0 \).

The precessional frequency of a nucleus (\( \omega \)) is given by equation [2-15]. Therefore, for an inhomogeneous \( B_0 \), nuclei experiencing different magnetic field strengths have different precessional frequencies. Magnetic field inhomogeneities are most pronounced at interfaces between tissues of dissimilar magnetic susceptibilities. As a result of the discrepancy in the magnetic field strengths within each tissue, a magnetic field gradient exists at the interface between the tissues. Nuclei that experience the gradient are dephased by an amount proportional to the magnitude of the gradient. For inhomogeneity gradients in the slice select direction, the extent of dephasing is given by equation [3-2].

46
\[ \Delta \phi = \gamma \cdot G_i \cdot \Delta r \cdot \text{TE} \tag{3-2} \]

where \( G_i \) is the magnitude of the gradient, \( \Delta r \) is the thickness of the imaging slice and TE is the echo time of the T2*-weighted imaging sequence.

The T2* effect has been used in vivo to study processes that involve changes in magnetic field inhomogeneities. Such studies include; identification of calcified lesions in the heart \(^1\), identification of haemorrhage \(^3\), determination of cerebral blood volume \(^4\), investigations of neuronal activity \(^5\), identification of tissue iron overload \(^6\), investigations of trabecular bone disorders \(^7\) and identification of Parkinson’s disease by cerebral iron depositions \(^8\). Of these, studies of neuronal activity are of particular relevance to the material presented in this thesis. At present, the exact mechanism behind T2* weighted signal intensity during neural activation remains unclear. In chapter 5 we present results illustrating the dependence of T2* weighting upon cerebral deoxyhaemoglobin concentration.

### 3.1.2 T2*-weighting

A gradient echo sequence is required to produce T2*-weighted images. The echo is not recalled by the application of a 180° pulse as with a spin echo sequence. Instead a reversal of the frequency encoding gradient is used to rephase the spins. Fig \([3-1]\) illustrates a simple gradient echo sequence. The corresponding K-space trajectory is given in fig \([3-2]\).

A low angle, slice selective RF pulse is applied (period 1: fig \([3-1]\), fig \([3-2]\)). Following slice selection both phase and frequency encoding gradients are applied (period 2: fig \([3-1]\), fig \([3-2]\)). The echo is then recalled by a readout frequency encoding gradient of opposite sign and twice the duration of the initial frequency encoding gradient (period 3: fig \([3-1]\), fig \([3-2]\)). The presence of macroscopic susceptibility induced magnetic field inhomogeneities affects the ability of the readout gradient to rephase the spins at TE, thereby reducing signal amplitude.
3.2 Diffusion

3.2.1 Physiological Significance of diffusion.

In vivo diffusion of water may prove to be of considerable physiological importance. Results presented in this thesis show that cerebral tissue water ADC (apparent diffusion coefficient) falls rapidly following a hypoxic-ischaemic episode (chapter 4). The exact mechanism behind ADC changes remains controversial. Three possible explanations have been proposed.

1. The **BOUND** model. Tissue water ADC decreases because of a net movement of water from the extracellular (high diffusion) to the intracellular space (low diffusion environment), as in cytotoxic oedema. Macromolecular surfaces within the intracellular space further restrict water diffusion.

2. The **OBSTRUCTION** model. Reduction in tissue water ADC is caused by an increase in tortuosity in the path of diffusing extracellular water. Increased tortuosity is a direct consequence of cytotoxic oedema causing a constriction of the extracellular space.

3. The **BULK PHASE** model. In living cells, subcellular organelles and structures aid the water mobility by active transport processes. A decrease in intracytoplasmic macromolecular motion and an increased intracellular viscosity act to reduce ADC of water.

The most favoured explanation is a combination of the “bound” and “obstruction” effects. However, studies using $^{133}$Cs as an intracellular diffusion marker, have shown a decreased intracellular ADC following ischaemic injury. Additionally, investigations into the diffusion of intracellular metabolites have encountered similar findings. Such evidence suggests that the third effect may, at least, be partially responsible for the decline in ADC following ischaemia.
3.2.2 Diffusion weighting

The transverse magnetisation of randomly moving spins experiencing a magnetic field gradient becomes irreversibly dephased. This results because the resonance frequency of moving spins in a magnetic field gradient changes along the direction of the gradient. Such an effect may be used to study the diffusion of tissue water molecules in vivo.

A diffusion weighted sequence can be created by insertion of 'diffusion sensitising' gradients on both sides of a 180° pulse, in either a spin-echo or a stimulated echo (fig [3-3]) sequence. After the application of the first diffusion gradient, static spins along the gradient direction have acquired a phase angle; dependent upon the duration of the gradient and the position of the spins within the gradient. A second diffusion gradient, identical to the first is then applied. As the spins have received a 180° pulse prior to the application of the second diffusion gradient; the second gradient simply removes any phase angle that had been acquired by static spins due to the first diffusion gradient. Therefore, the pair of diffusion sensitising gradients has had no effect upon the phase of static spins. However, for spins that are randomly moving during the application of the diffusion gradients and, during the time period between the two diffusion gradients, the effects of the two diffusion gradients do not cancel. The phase angle accumulated by moving spins is no longer just dependent upon the location of the spins within the gradient and the duration of the gradient, but also reflects the distance moved by the spins during the experiment.

The attenuated signal amplitude is given by equation [3-3].

\[
S = S_0 \exp\left(\frac{-TE}{T_2}\right) \exp\left(\frac{-\gamma^2 G^2 \delta^2 (\Delta - \frac{\delta}{2})D}{12}\right) \tag{3-3}
\]
where S is the attenuated signal, $S_0$ is the signal immediately following the 90° excitation pulse, TE is the echo time of the sequence, G is the gradient strength, $\Delta$ is the time interval separating the onset of the two diffusion gradients and $\delta$ is the duration of each diffusion gradient pulse. D is the diffusion coefficient of water measured in m²s⁻¹.

### 3.3 Phosphocreatine and inorganic Phosphates

#### 3.3.1 Physiological Significance of Phosphates

Phosphates are an extremely important component of energy metabolism in the brain (fig [3-4]). A continuous supply of energy is necessary for brain function. Oxidative phosphorylation is the main source of energy for neurones. A large proportion of the energy is supplied by glucose oxidation and converted into a chemical store as adenosine triphosphate (ATP). ATP contains three phosphate groupings and as such is a major carrier of energy in the brain. Upon hydrolysis to adenosine diphosphate (ADP) and $P_i$, a large amount of energy is liberated.

\[
[\text{ATP}^d] + [\text{H}_2\text{O}] \rightarrow [\text{ADP}^3] + [\text{HPO}_4^{2-}] + [\text{H}^+] + \Delta G \tag{3.4}
\]

where $\Delta G$ is the energy released by this reaction.

Increased breakdown of ATP causes an accumulation of ADP and $P_i$. A steady and rapid production of ATP is required to maintain ATP levels. Creatine is phosphorylated by creatine kinase, producing phosphocreatine (PCr), from which ATP can be rapidly generated.

\[
\text{Phosphocreatine} + \text{Adenosine Diposphate} + H^+ \rightarrow \text{Adenosine Triphosphate} + \text{Creatine} \tag{3.5}
\]
Therefore the ratio \([\text{PCr}] / [\text{Pi}]\) can be used as an index of cellular energy metabolism. This has been used in studies of hypoxia-ischaemia in the newborn\textsuperscript{11,12} as a predictor of brain injury and neurodevelopmental outcome.

### 3.3.2 $^{31}$P Spectra

A typical $^{31}$P brain spectrum obtained from the parietal lobes of a newborn piglet is shown in fig \[3-4\]. The peaks from left to right correspond to (1) phosphomonoesters, (2) inorganic phosphate, (3) phosphodiesters, (4) phosphocreatine, (5) $\gamma$-nucleotide triphosphates, (6) $\alpha$-nucleotide triphosphates, (7) $\beta$-nucleotide triphosphates.

Resonance areas are measured automatically by Lorentzian-profile, gradient search $\chi^2$ minimisation in the frequency domain, with prior knowledge (number of resonances, starting values for the chemical shifts, widths and amplitudes, and nucleotide triphosphate (NTP) multiplicities ($J$ coupling 16 Hz)). The ratio \([\text{PCr}] / [\text{Pi}]\) can then be determined.
3.4 References


Fig [3-1]  Pulse and gradient timing diagram indicating the slice selective RF pulse, phase and frequency encode gradients necessary to generate an echo.

Fig [3-2]  K-space trajectory depicting a single repetition of the gradient echo pulse sequence illustrated in fig [3-1].  A, B, C and D correspond to time points A, B, C and D in fig [3-1].
Pulse sequence diagram of a stimulated echo diffusion weighted sequence, indicating the additional gradients necessary to cause diffusion weighting. TE is the echo time, TM is the mixing period, $\delta$ is the duration of the diffusion gradients and $\Delta$ is the time between the leading edges of the pair of diffusion gradients.
Fig [3-4] Schematic diagram illustrating oxidative phosphorylation. ATP is adenosine triphosphate and ADP is adenosine diphosphate.

Fig [3-5] \(^3\)P spectrum obtained from the parietal cortex of a newborn piglet. Resolvable peaks are numbered left to right. (1) phosphomonooesters, (2) inorganic phosphate, (3) phosphodiesters, (4) phosphocreatine, (5) \(\gamma\)-nucleotide triphosphates, (6) \(\alpha\)-nucleotide triphosphates, (7) \(\beta\)-nucleotide triphosphates.
CHAPTER 4

Quantitative ADC and $T_2$
Measurements in the Piglet Model of
Secondary Energy Failure
CHAPTER 4

Quantitative ADC and $T_2$ Measurements in the Piglet Model of Secondary Energy Failure

4.1 Introduction

Hypoxic-ischaemic brain injury arising in the perinatal period affects between four and eight per 1000 full term births per year in the UK. The aim of this work is to use NMR as a non-invasive measurement technique to investigate perinatal brain development in normal infants and those that have suffered hypoxic-ischaemic (H-I) brain injury.

Since 1982 the group has studied over 150 newborn infants with clinical evidence of acute perinatal asphyxia, using $^{31}$P and $^1$H MRS. Marked increases in the cerebral concentrations of inorganic phosphate and lactate ($[P_i]$ and $[\text{lactate}]$ respectively), and reductions in phosphocreatine concentration [$PCr$] and nucleotide triphosphate concentration [$NTP$] (mainly ATP), have been quantified within specified brain regions in infants that had suffered an acute period of hypoxia-ischaemia, indicating failure of energy metabolism. In infants thought to have been subjected to an acute intrapartum insult there was usually a latent period of up to 15 hours before impairment of energy metabolism could be detected. This phenomenon was termed "secondary" or "delayed" energy failure. The delayed changes detected by MRS, especially the fall in $[PCr]/[P_i]$ ratio, were strongly predictive of death, or survival with reduced brain growth and severe neurodevelopmental disabilities, allowing the prognosis for long-term outcome to be assigned with reasonable precision, within a few days of birth. The presence of a latent period before energy failure developed suggested that cerebroprotective interventions following resuscitation might have the potential to reduce or prevent delayed energy failure and the permanent brain injury with which it is associated.
Accumulating evidence points to a biphasic pattern of brain cell injury following a transient episode of severe hypoxia-ischaemia in the developing brain, with distinct early and delayed phases. However, the mechanisms underlying this pattern of injury remain unclear. Early neuronal injury is probably due largely to intracellular calcium toxicity, membrane failure and the effects of glutamate excitotoxicity (possibly mediated by the generation of nitric oxide). However, delayed cell injury appears to involve different mechanisms and there is some evidence to suggest that the process of programmed cell death (apoptosis) is involved.

In order to test the efficacy of cerebroprotective strategies, a neonatal animal model has been developed which is capable of reproducing the development of delayed energy failure after resuscitation following a hypoxic-ischaemic episode. Using newborn piglets, the biphasic pattern of cerebral energy impairment has been reproduced following an acute reversed hypoxic-ischaemic insult. The severity of delayed energy failure varies between individual animals, presumably due to biological variability in the metabolic and physiological responses to hypoxia-ischaemia. However, a strong relationship has been demonstrated between the severity of the acute energetic disturbance (derived from the time integral of NTP depletion) and the severity of delayed energy failure (particularly minimum [PCr]/[Pi]), indicating a clear dose-response effect.

MRI measurements of apparent diffusion coefficient (ADC) and T2 of brain tissue water have proved uniquely sensitive in the delineation of ischaemic injury. Compared with MRS, diffusion weighted imaging (DWI) has the advantage that it provides a high spatial resolution in the form of quantitative maps of ADC of brain water. ADC values have been shown to decline significantly within minutes of the onset of an ischaemic episode. DWI shows promise in several clinical applications, notably in the assessment of stroke patients, and, germane to the subject of this chapter, in the study of perinatally asphyxiated infants. Although the mechanisms for this change in ADC values during, and subsequent to, cerebral ischaemic damage have not been unambiguously established, the redistribution of some water from the
extracellular (high diffusion) environment to the intracellular (low diffusion) environment could change the average diffusion value, and might also reduce the diffusion coefficient in the reduced extracellular environment by increasing the barriers to water motion (the tortuosity of the water). Ionic homeostasis is normally preserved by the sodium / potassium pump and this function is responsible for a large proportion of cerebral energy consumption. During ischaemia, high energy phosphates are depleted leading to cell swelling through an osmotically driven influx of water into cells, associated with an increase in intracellular sodium concentration. Therefore, during ischaemia it is anticipated that changes in both ADC values and concentrations of high energy phosphates, as measured by $^{31}$P MRS, should occur almost simultaneously. However, such a relationship does not necessarily exist during the process of secondary energy failure that occurs in the re-oxygenated brain following H-I injury.

Measurements of $T_2$ in adult rat brain have shown nearly a 100% increase in $T_2$ values during a period of 24 hours followed by a return to normal values after 168 hours as a consequence of permanent surgical occlusion of the middle cerebral artery. Changes in $T_2$ are thought to be related to an increase in the total amount of water, or a change in the bulk to bound water fraction. Brain swelling occurs as a result of vasogenic oedema following transient ischaemia. Early changes in $T_2$ are detectable and may prove to be of clinical importance.

The aim of this work was to investigate the relationship between changes in cerebral ADC values and relative high energy phosphate concentrations during secondary energy failure, in order to establish DWI as a high resolution technique for monitoring cerebral hypoxic-ischaemic brain injury. A further aim was to investigate the time course of DWI and $T_2$ changes during secondary energy failure, in order to find an early marker for secondary energy failure.

The established porcine model of perinatal asphyxia was used in the study, in which $^{31}$P MRS spectra acquired from the same animals were employed as an index of cerebral energy failure. It has been shown that this model faithfully reproduces the
sequence of alterations in cerebral energy metabolism that are observed in perinatally asphyxiated human infants. During hypoxia-ischaemia, $^{31}$P MRS shows falls in the levels of the high energy metabolites PCr and adenosine ATP concomitant with a rise in $P_i$. Initial recovery immediately post-resuscitation, is then followed by gradual declines in [PCr] and [ATP] and a large rise in $[P_i]$ over the next 48 hours; the latter changes constitute “secondary” or delayed energy failure. [PCr]/[$P_i$] is proportional to the “phosphorylation” potential, and following perinatal asphyxia, low values convey an unfavourable prognosis.

In the present study, baseline ADC and $T_2$ data were obtained from cortical grey and sub-cortical white matter in the piglet brain, and changes in ADC and $T_2$ values were then investigated up to 48 hours following a defined transient hypoxic-ischaemic episode. The latter measurements are of clinical relevance to MRI examinations of perinatally asphyxiated infants who are routinely scanned in our hospital within a few days of birth.

4.2 Methods

4.2.1 Animal Model

Experiments were performed on nine healthy term Large White piglets, aged less than 24 hours. Anaesthesia was induced by inhalation of 5% isoflurane followed by tracheotomy and ventilation with a mixture of $N_2O$, $O_2$ and isoflurane (<1.5%). Inflatable cuffs were positioned around both common carotid arteries. The anaesthetised animal was then positioned prone on a temperature-regulated water-filled mattress in a cylindrical perspex pod, designed to fit snugly into the bore of the magnet. The head was constrained using a stereotactic system, with care being taken to ensure precise positioning of both the animals within the pod and the pod within the magnet bore. Heart rate, blood pressure, rectal and tympanic temperatures were monitored continuously.
Transient cerebral hypoxia-ischaemia was induced as previously described. Briefly, the inspired oxygen concentration was reduced to 12%, and both common carotid arteries were temporarily occluded until severe depletion of high energy phosphates was detected by $^{31}$P MRS. The animals were then resuscitated by increasing the inspired oxygen to normalise the arterial partial pressure of O$_2$ (PaO$_2$) and releasing the carotid occluders. The animals were maintained with full intensive care inside the magnet bore as observations continued throughout the subsequent 48 hours.

4.2.2 MRI and MRS

NMR measurements were performed using a 7 T Bruker Biospec spectrometer system (Bruker, Karlsruhe, Germany). Data were acquired using a double-tuned ($^{31}$P and $^1$H) 25 mm diameter surface coil for radiofrequency (RF) transmission and reception. The coil was positioned on the intact scalp over the parietal lobes of the brain.

Fully relaxed $^{31}$P pulse-acquire spectra were obtained with a relaxation delay of 10 seconds. Resonance peaks were measured by Lorentzian curve fitting. During the insult, energy status was monitored using 36 summed free induction decays (FIDs) and, post-resuscitation, 384 FIDs were summed to obtain a more accurate measurement.

ADC values were measured in a coronal plane using a diffusion-weighted stimulated echo acquisition (STEAM) sequence (Meboldt et al, 1991). The sequence timings were as follows: repetition time TR = 2 s, TE = 34 ms, mixing time TM = 200 ms, acquisition time = 2.2 minutes per image. The slice thickness was 2.5 mm, the field of view was 3 cm and the image matrix of 128x64 was zero-filled to 128x128 to give an in plane resolution of 0.23 mm.

Rectangular diffusion-sensitising gradient pulses were applied between the first and second RF pulses, and after the third RF pulse of the STEAM sequence. The
duration of each diffusion-sensitising gradient pulse was 10 ms and the separation between the rising edges of the two gradient pulses was 214 ms. Gradient strengths of 0, 17, 25 and 30 mT.m\(^{-1}\) were employed giving ‘b factors’ of 0, 440, 900 and 1350 \(\times 10^9\) m\(^2\).s\(^{-1}\) for diffusion sensitisation along the x and y directions and 0, 724, 1270 and 1780 \(\times 10^9\) m\(^2\).s\(^{-1}\) along the z axis after accounting for cross-terms arising from the slice selection (z) gradients. ADC maps were calculated using a log-linear fit algorithm. Average non-directional diffusion maps (ADC\(_{av}\)) were then obtained by averaging the ADC value from all three axes. This eliminates the confusing effects of diffusional anisotropy that is predominantly present in white matter. An average diffusion value for all brain tissue within the field of view of the surface coil was obtained by removing signal from cerebro-spinal fluid (CSF), scalp and low signal to noise ADC values, by use of an image segmentation algorithm. In this manner an globally averaged non directional ADC value was obtained that has been derived from approximately the same volume of brain tissue examined by \(^{31}\)P MRS and allowed direct comparison between the two measurements.

\(T_2\) values were measured from the same imaging slice using a standard spin echo acquisition sequence. The sequence timings were as follows: TR = 2 s, TE = 35 80 120 and 160 ms, acquisition time = 2.2 minutes per image. The slice thickness was 2.5 mm, the field of view was 3 cm and the image matrix of 128x64 was zero filled to 128x128 to give the same resolution as for DWI. \(T_2\) maps were generated by obtaining the gradient of the natural logarithm of normalised signal intensity for each pixel versus echo time and the same post processing carried out to remove unwanted CSF and scalp signal, as for ADC\(_{av}\) maps.

To assess the spatial variation of changes in \(T_2\) relaxation rate following the acute insult, horizontal profiles were taken at a fixed vertical position for every \(T_2\) map from each individual animal. Profiles for a single animal were placed sequentially, in order of time. Thus, a contour map of the profiles was produced. A typical example is illustrated in fig [4-1]. The vertical axis shows the scan number of each profile, and is arranged in order of time. The horizontal axis represents the pixel
position for each point within the profiles. The grey scale represents the $T_2$ value at a particular location in space and time.

Upon examination of a $T_2$ profile, the region of the profile containing the gyrus is seen to have a characteristic shape (fig [4-2]). Typically, extremely high $T_2$ values are seen on either side of a gyrus (regions A, fig [4-2]); these represent the sulci, containing CSF. Immediately next to these sulci (regions B, fig [4-2]), there is a trough in $T_2$ values, which we attributed to the thin band of cortical grey matter. Finally, at the centre of the gyrus (region C, fig [4-2]), the $T_2$ value is once again elevated and forms a peak. Region C is identified as subcortical white matter. This pattern may be recognised within the grey scale contour maps at scan 1, for both medial (fig [4-1], pixels 43-54) and lateral (fig [4-1], pixels 62-70) gyri. The variation of this pattern over time for a particular gyrus may be followed using the contour map, along the vertical scan axis (fig [4-1]). Because the characteristic pattern within a gyrus is used to identify the region of interest (fig [4-2]), the use of contour maps makes it possible to unambiguously assign grey and white matter regions even though brain swelling may occur over time, or the animal may have moved fractionally between scans. A similar analysis procedure was used to investigate regional changes in $ADC_{av}$ values.

As a precursor to data analysis, piglets which did not show, or showed very mild, signs of SEF were eliminated from statistical calculations. Piglets whose $[PCr]/[P_i]$ did not fall below 0.5 during the 48 hours following hypoxia-ischaemia were excluded. From the nine piglets studied two piglets were excluded based on this criteria.

4.3 Results

Fig [4-3] (a) shows a typical change in $[PCr]/[P_i]$ ratio during and upto 48 hours following transient hypoxia-ischaemia. Figs [4-3] (b) and (c) show changes in global $ADC_{av}$ and $T_2$ respectively for the same animal during this period. Global
ADC\textsubscript{av} was found to be fractionally lower than the baseline value at four hours following the insult. During the following 48 hours global ADC\textsubscript{av} fell gradually to reach approximately 66% of the baseline value at 46 hours post ischaemia. T\textsubscript{2} values remained close to baseline values up till 12 hours following the insult. Whereupon, there was a linear increase of T\textsubscript{2} value with time. T\textsubscript{2} values at 46 hours following the insult were elevated by approximately 50% over the baseline T\textsubscript{2} value. Due to time constraints, ADC\textsubscript{av} and T\textsubscript{2} could not be measured during the hypoxic-ischaemic episode.

Fig [4-4] (a) illustrates the mean [PCr]/[P\textsubscript{i}] ratio at five time points following the insult (n = 7). There was no significant difference between baseline [PCr]/[P\textsubscript{i}] and the 4 hour and 12 hour time point as determined by the two tailed paired Student's t-test. However, at 24 and 48 hours the [PCr]/[P\textsubscript{i}] ratio was significantly different from the baseline value (p < 0.05). Fig [4-4] (b) shows mean whole brain ADC\textsubscript{av} values at the same five time points following the insult (n = 7). The baseline ADC\textsubscript{av} value was 0.91 ± 0.03 (1 SD), compared with 0.86 ± 0.05 at 4 hours post resuscitation, 0.75 ± 0.09 at 12 hours, 0.61 ± 0.07 at 24 hours and 0.55 ± 0.04 at 48 hours post-resuscitation (x 10\textsuperscript{-9} m\textsuperscript{2}.s\textsuperscript{-1}). There appears to be a small, but statistically significant difference between the baseline global ADC\textsubscript{av} value and global ADC\textsubscript{av} value measured at 4 hours post resuscitation (p < 0.05). There was also a significant change from baseline in global ADC\textsubscript{av} at the 12 hour, 24 hour and 44 hour time points (p < 0.05). The data suggests that the small decline in global ADC\textsubscript{av} in the acute period following hypoxia-ischaemia is measurable and could reflect the magnitude of early neuronal injury. This initial decrease could be of prognostic value in determining the eventual severity of hypoxic-ischaemic injury.

Fig [4-4] (c) shows mean whole brain T\textsubscript{2} values (n = 7). Baseline T\textsubscript{2} values were 60.5 ± 1.0 (SD) ms, compared with 61.7 ± 1.9 ms at 4 hours following the insult. There was no significant difference in whole brain T\textsubscript{2} between the baseline and 4 hour time points. However, at 12, 24 and 44 hour post resuscitation there was a significant difference from the baseline value (p < 0.05).
Fig [4-5] shows a time series of ADCav maps obtained in a single neonatal piglet following H-I insult. The field of view of the surface coil extends to a depth of approximately 1 cm into the piglet brain and is sufficient to encompass several gyri. The maps show a general pattern of ADCav reduction (darkening of maps), with marked variations in time course between different anatomical regions. Parasagittal cortical grey matter regions show the earliest declines in ADCav and are responsible for much of the difference seen in the map obtained 22 hours post-resuscitation. This early decline may reflect the varying sensitivity to damage of different brain regions and demonstrates the high regional sensitivity of DWI measurements. At 45 hours, ADCav values from all brain regions were markedly reduced.

Fig [4-6] shows a similar time series of T2 maps for the same animal as depicted in fig [4-5]. Following the insult, there is a gradual rise in the global T2 value, represented by a brightening of the T2 maps. Upon closer examination there appears to be a change in contrast between grey and white matter with time, indicating a different rate of rise of T2 in these two brain tissue types. The maps indicate contrast between grey and white matter in the baseline maps. The contrast appears to gradually disappear, and by 25 hours post resuscitation there appears to be little difference in T2 between white and grey matter. However, by 45 hours post resuscitation an appreciable difference between white and grey matter T2 is again observable. In contrast to the ADCav maps in fig [4-5], the progression of the injury from lateral to medial gyrus is not as clearly discernible on the T2 maps.

Fig [4-7] shows the spatial variation of mean T2 values in cortical grey and white matter regions, in the medial and lateral gyri during the 48 hours following the hypoxic-ischaemic insult (n = 7). There was no statistically significant difference in grey matter T2 values between medial and lateral gyri until 16 hours following the insult. Similarly, there was no statistical difference between medial and lateral white matter T2 values until 16 hours following the insult. After the 16 hour time point, the graph indicates a divergence in the response of lateral and medial gyri. Lateral gyri T2 values for both grey and white matter increase more rapidly than T2 values for grey and white matter in the medial gyrus between 16 and 30 hours. A statistically
significant difference is present between medial and lateral gyri grey matter T2 values at the 24 and 28 hour time points (p < 0.05). Lateral and medial gyri white matter T2 values were also found to be significantly different at 24 hours (p < 0.05), but this difference failed to reach significance at 28 hours (p = 0.06). Further experimentation may be necessary to statistically validate this difference. Additionally, during the 48 hours following the insult, white matter T2 values were generally found to be statistically greater than grey matter T2 values (p < 0.05).

Fig [4-8] shows mean ADCav values for medial and lateral grey matter regions (n = 7). There was no significant difference between medial and lateral grey matter ADCav values at the baseline and 4 hour time points. However, ADCav values in lateral grey matter seem to decrease earlier than medial grey matter (see fig [4-6]), and a statistically significant difference between these regions was observed at 12 and 16 hours post resuscitation (p < 0.05). Medial and lateral white matter regions did not show a significant difference in response to hypoxia-ischaemia. Medial white matter ADCav values were generally significantly (p < 0.05) greater than medial grey matter values during the 48 hours following the insult. Lateral white matter ADCav values were also found to be significantly greater than lateral grey matter values at the majority of time points following the insult (p < 0.05).

Fig [4-9] illustrates the relationship between global ADCav and [PCr]/[Pi] ratio. Fig [4-9] shows global ADCav values from 7 animals measured at different time points and plotted against the [PCr]/[Pi] index measured during the same experiment. It is hypothesised that changes in ADCav during secondary energy failure should follow changes in [PCr]/[Pi]. The linear regression on fig [4-9] was used to test this hypothesis. The strong correlation coefficient (r = 0.95), indicates support for the above hypothesis. Linear correlation coefficients for individual animals were found to be 0.94 < r < 0.99. A similar plot of whole brain T2 versus [PCr]/[Pi] is illustrated in fig [4-10] (n = 7). The graph indicates a biphasic relationship between [PCr]/[Pi] and T2. Changes in T2 were observed to occur with changes in [PCr]/[Pi]. However, when the [PCr]/[Pi] ratio was zero and could change no further, T2 changes continued.
This suggests that cell destruction continues to occur after energy metabolism has completely collapsed.

4.4 Discussion

4.4.1 Monitoring H-I Brain Injury using ADC and $T_2$ Maps

We have demonstrated a reduction in ADC values associated with a decline in energy metabolism following transient cerebral hypoxia-ischaemia in the newborn piglet. ADC maps are capable of detecting variations in cerebral injury between brain regions at a spatial resolution that is not available using current $^{31}$P MRS techniques. Changes in ADC values were found to be correlated with $[\text{PCr}]/[\text{Pi}]$ ratio changes in this animal model, and may provide an alternative method of detecting delayed neuronal damage. $^{31}$P MRS can only be performed in high field magnets (greater than 1.5 Tesla), and even at the field strength of 7 Tesla employed in the present study, necessitates a long examination time (approximately 40 minutes). The ADC value should be constant when measured at the different field strengths commonly employed in commercial MR scanners, which typically vary between 0.5 Tesla (or less) and 3 Tesla. However, both MRS and MRI procedures are non-invasive and allow repetitive measurements to be made, which may be necessary in determining the severity of H-I injury during the 48 hours following birth. A potential problem with ADC measurements is the extreme sensitivity of the experiment to subject motion. This may be overcome by either using high-speed imaging techniques, such as echo-planar imaging or by use of a motion-tracking and correction procedure.

We have also demonstrated a rise in $T_2$ over the 48 hours following the ischaemic injury. $T_2$ examinations are easy to perform and are routine procedures in most clinical MRI centres. However, $T_2$ varies with magnetic field strength, hence it may not be possible to compare data from different centres. Additionally, although there is a rise in $T_2$ value following ischaemia, $T_2$ changes are not intrinsically linked to energy metabolism and represent the end point of ischaemic damage, oedema and
cell destruction. $T_2$ can be used following ischaemic injury to produce spatially
detailed maps depicting the extent of brain injury and to follow the spatial distribution
of developing injury.

$ADC_{av}$ maps clearly show the pattern of cerebral injury. There is an early
darkening of the lateral cortical grey matter, representing a fall in $ADC_{av}$. This
darkening pattern gradually progresses medially. This effect is not seen as clearly in
$T_2$ maps, however, evidence for its existence is suggested by the delayed rise in $T_2$
values in the medial gyrus (fig [4-7]). The parasagittal injury is thought to reflect
differences in the blood supply to cortical brain regions.

4.4.2 $ADC_{av}$ and $T_2$ as predictive markers of Cerebral Injury

A window exists between the primary ischaemic episode and the onset of cell
death during secondary energy failure. During this time, treatment to limit the
damaging effects of secondary energy failure may be administered (e.g. MgSO$_4$,
hypothermia and / or cerebroprotective drugs). It is important to be able to select
those individuals that are susceptible to secondary energy failure at an early stage for
administration of the treatment. From the data gathered, global $ADC_{av}$ values 4 hours
after the ischaemic episode show a decline from baseline in the animals that undergo
secondary energy failure. This was found to be statistically significant ($p<0.05$). The
magnitude of the fall in $ADC_{av}$ may be predictive of outcome. However, upon
regional examination of $ADC_{av}$ (medial and lateral gyri), the difference from baseline
of $ADC_{av}$ was not statistically significant. Further work needs to be done to
statistically validate this difference.

There was no significant change from baseline in global $T_2$ values immediately
following the insult. Closer examination of the data, by separating medial grey-white
matter and medial-lateral gyri reveal subtle differences that are masked by whole brain
$T_2$ measurements, thus indicating the different susceptibilities of various brain tissue
to ischaemic injury. These early changes in $T_2$ are thought to be a result of vasogenic
oedema, and are not believed to be predictive of secondary energy failure.
Further investigation using MRI techniques in this model should be able to statistically validate the significance of imaging techniques in providing early markers of brain injury. Also, MRI techniques might aid the understanding of the spatial pattern of brain injury, which may help in predicting neurodevelopmental outcome and specific disabilities in children that suffer from H-I brain injury. Additionally, the ability to monitor delayed neuronal damage in this piglet model will be invaluable in assessing the efficacy of various proposed neuronal rescue strategies. Furthermore, the ability to perform the same measurements in infants should aid in patient selection and therapy monitoring when these procedures are applied to humans.
4.5 References


Contour Map produced by arranging $T_2$ profiles in order of time.

Fig [4-1]  Grey scale $T_2$ contour map depicting changes in $T_2$ values along a profile with scan number following an hypoxic ischaemic insult. (Average time between scans = 4 hours).
Fig [4-2] Diagram indicating the profile analysis procedure used to investigate spatial variations in ADC and T1 maps.
Fig [4-3]  (a) Graph showing typical changes in [PCr]/[P_i] ratio for a single animal following a hypoxic-ischaemic insult at time = 0. (b) Graph showing typical changes in global ADC_{av} for the same animal as in (a). (c) Graph showing typical changes in global T_2 for the same animal as in (a).
Fig [4-4] Graphs showing average [PCr]/[P], global ADC$_{av}$, and global $T_2$ ((a), (b) and (c) respectively) at baseline and four time points following a hypoxic-ischaemic insult at time = 0, (n = 7). Error bars represent 1 SD.
Fig 4-5 A typical time series of ADC$_w$ maps obtained from an individual neonatal piglet following an acute hypoxic-ischaemic insult.
Fig [4-6] A typical time series of $T_2$ maps obtained from an individual neonatal piglet following an acute hypoxic-ischaemic insult.
Fig [4-7] Spatial variations of $T_2$ changes following a hypoxic ischaemic insult at time $= 0$, ($n = 7$).
Fig [4-8] Spatial variations of $\text{ADC}_{av}$ changes following a hypoxic-ischaemic insult at time $= 0$, $(n = 7)$. 
Fig [4-9]  Relationship between [PCr]/[P_i] changes and ADC_{av} changes that occur during secondary energy failure, (n = 7, r = 0.95).
Fig [4-10] Relationship between [PCr]/[P,] changes and global $T_2$ changes that occur during secondary energy failure, ($n = 7$).
CHAPTER 5

MRI Measurements of Cerebral Deoxyhaemoglobin Concentration. A Correlation with Near Infra Red Spectroscopy.
CHAPTER 5

MRI Measurements of Cerebral Deoxyhaemoglobin concentration [dHb] - Correlation with Near Infra Red Spectroscopy (NIRS).

5.1 Introduction

Magnetic field inhomogeneities in biological tissues, may be caused by macroscopic effects (e.g. field gradients at interfaces between substances of different magnetic susceptibility \( \chi \), i.e. around air cavities in the body), or by microscopic sources of magnetic disturbance. Microscopic effects reflect the concentration of magnetic materials within tissue, and have been used to provide measures of iron content in the brain \(^1\), and changes in the concentration of cerebral deoxyhaemoglobin [dHb] \(^2\). The magnetic resonance signal is made sensitive to these magnetic effects by using an imaging experiment that provides contrast related to the spin-spin relaxation time in the presence of field inhomogeneities (\( T_2^\ast \)). Measurement of cerebral [dHb] provides a MRI contrast mechanism that is related to tissue function (e.g. blood oxygenation), and may further the utility of MRI in delivering prognostically useful information.

Gradient echo pulse sequences do not contain a 180° ‘refocussing’ pulse and are sensitive to the presence of magnetic disturbances. Thus, gradient echoes are \( T_2^\ast \) weighted. In order to quantify the \( T_2^\ast \) relaxation time, a series of increasing echo times (TE’s) are used to produce images with increasing \( T_2^\ast \)-weighting. If both \( T_2 \) and \( T_2^\ast \) relaxation times are measured, then the value for \( T_2^\ast \) can be calculated using equation [5-1]. It is believed that \( T_2^\ast \) may be a more sensitive measure of magnetic field inhomogeneities than \( T_2^\ast \).

\[
\frac{1}{T_2^\ast} = \frac{1}{T_2} + \frac{1}{T_2^\ast} \quad [5-1]
\]
Expressed in terms of the respective relaxation rates, equation [5-1] can be rewritten as

\[ R_2^* = R_2 + R_2' \]  

where \( R_2 = 1/T_2, R_2^* = 1/T_2^* \) and \( R_2' = 1/T_2' \).

NIRS is an established method for the in vivo measurement of changes in \([dHb]\) and the concentration of oxyhaemoglobin \([HbO_2]\). The method relies on the principle that changes in oxy/deoxyhaemoglobin concentrations precipitate detectable fluctuations in the quantity of light absorption at the characteristic wavelength of these chromophores. The NIRS method is non-invasive but, applied in-vivo, suffers from the fundamental problem of light scattering. This makes the determination of absolute concentrations of oxy/deoxyhaemoglobin difficult. However, the problems of light scattering can be partly resolved by the use of second differential spectroscopy. Absolute concentrations can then be determined by reference to a constant internal standard. As the concentration of water in tissue is stable and known to within an accuracy of 1%, water can be used as this reference. This technique has been used to measure absolute \([dHb]\) in a variety of animal models and in the neonatal human brain.

In this study, the relationship between \( T_2^* \) relaxation time and absolute \([dHb]\) was investigated, by simultaneous MRI and NIRS measurements. This study did not attempt to measure BOLD signal behaviour, instead it concentrated purely upon observing the effects of increasing \([dHb]\) on the absolute \( T_2^* \) relaxation time. After quantitation of \( T_2^* \) signal behaviour in response to changes in \([dHb]\), it is hoped that it may become possible to produce maps of cerebral \([dHb]\) by MRI, which could be of value in determining regions of brain with abnormal \( O_2 \) delivery or consumption. Such abnormalities are thought to occur during hypoxic-ischaemic brain injury, during which blood supply / oxygen delivery to part or all of the brain is insufficient to maintain energy metabolism.
5.2 Method

Experiments were carried out using a newborn piglet model as developed in our laboratory. The model has been used in studies of cerebral energy failure, and is fully described in previous literature. Seven Large White piglets, less than 24 hours old, were investigated using simultaneous MRI and NIRS. An intramuscular injection of midazolam (0.2 mg kg\(^{-1}\)) was used for sedation, and anaesthesia was initially induced using 5% isoflurane. A tracheotomy was carried out and anaesthesia was then maintained by ventilation with a mixture of isoflurane (<1.5%), \(\text{N}_2\text{O}\) and \(\text{O}_2\). \(\text{FiO}_2\) was measured using an oxygen electrode. Ventilator settings were altered to maintain the arterial partial pressure of carbon dioxide (\(\text{PaCO}_2\)) within the normal range (4-6 kPa) throughout the study. Cannulation of the umbilical artery and vein was performed using a 3.5 mm outer diameter polyvinyl catheter. During the course of the study, arterial blood was withdrawn and analysed for \(\text{PaO}_2\) and \(\text{PaCO}_2\), electrolytes and glucose. Continuous monitoring of rectal and tympanic temperature, blood pressure, heart rate and \(\text{SaO}_2\) was also performed.

After placing the animal in a specially constructed cylindrical perspex pod, a stereotactic holder was employed to constrain head movement during the examination. Rectal temperature was maintained between 38.5 and 39.0 °C, using a temperature regulated water-mattress positioned within the pod, beneath the animal. NIRS optodes were positioned over the intact skull, approximately 4 cm apart, above the parietal lobes. Non-magnetic black paint was applied to the skin over the skull to prevent reflected light re-entering the brain. Extraneous light was prevented from reaching the detector by lining the interior of the perspex pod with black cloth. For the MRI study, a 2.5 cm surface coil was positioned on the intact skull over the parietal lobes. The pod was then inserted into the bore of the magnet (7 Tesla / 20 cm bore magnet linked to a Biospec spectrometer, Bruker Instruments Inc.), and the position fixed until the end of the experiment. To further exclude extraneous light, black cloth was used to cover both ends of the magnet bore. One optode was connected to a broadband white light source.
and the other to a multi-wavelength (700-1000 nm) CCD spectrometer. The second differential technique was used to quantify absolute [dHb], by referencing the peak at 760 nm to the water features at 710 nm and 830 nm, assuming a fixed neonatal cerebral water content of 85% Changes in the concentration of HbO₂ and dHb were quantified by using their NIRS spectra with a spectral correction factor to account for the wavelength dependence of the optical pathlength.

At the end of each study the FiO₂ was reduced to zero (98% N₂O, 2% isoflurane), resulting in a large drop in [HbO₂] which stabilised after two to three minutes. Assuming that cerebral [HbO₂] is zero at this time, the absolute [HbO₂], [dHb] and total cerebral haemoglobin content (tc[Hb]) were then known. Back calculation was performed to determine absolute values of [HbO₂] for all points during the study, since absolute values of [HbO₂] cannot be obtained by the second differential technique. Assuming a constant haematocrit during the course of the experiment, then changes in tc[Hb] are directly related to changes in CBV.

Calculations using these methods resulted in values for [dHb] of 11 ± 3 mM (n = 7), and were not significantly different from values measured in the newborn human infant tc[Hb] was calculated to be 47 ± 14 mM (n = 7), consistent with expectations for neonatal brain.

The 2.5 cm surface coil was used for both RF transmission of pulses, and for signal detection. Prior to the commencement of imaging, the magnet was shimmed to obtain a half height linewidth of the water resonance of less than 36 Hz. T₂*-weighted MR imaging was performed using a FLASH sequence with an extended gradient echo time. Images were obtained with four echo times (TE = 5.2, 15, 25 and 34 ms, TR = 84 ms, slice thickness = 4 mm, field of view = 6 cm, image matrix = 256x128). After obtaining baseline images under non-hypoxic conditions, FiO₂ was decreased stepwise, causing a corresponding reduction in PaO₂, as measured by blood gas analysis. Four T₂*-weighted images were acquired at each FiO₂ step, in order to produce T₂* maps of the brain tissue within the field of view of the RF coil. T₂* maps were generated by
obtaining the gradient of the natural logarithm of normalised signal intensity for each pixel versus TE.

$T_2$-weighted MRI was performed using a standard spin echo experiment. Four images were obtained with increasing $T_2$-weighting ($TE = 35$ ms, $80$ ms, $120$ ms and $160$ ms, $TR = 2080$ ms, slice thickness $=5$ mm, field of view $= 6.00$ cm, image matrix size $= 64 \times 128$). $T_2$ measurements were performed at the beginning of the experiment, and after the animal had been sacrificed, for five of the seven animals. $T_2$ maps were produced using the same analysis procedure as used for $T_2^*$ maps.

For correlation with global measurements of $[dHb]$ a global $T_2^*$ value was obtained. This was determined from the $T_2^*$ maps obtained at each $FiO_2$ step. The maps were initially thresholded to remove background noise and high signal from CSF. Scalp signal was removed by image processing. An average value of the remaining pixels was used to obtain global $T_2^*$ values. The field of view of our surface coil was believed to approximately reflect the extent of tissue examined by NIRS.

To examine regional changes in $T_2^*$ more closely, both $T_2$ and $T_2^*$ maps were obtained at baseline and immediately after sacrificing the animal. This allowed calculation of $T_2'$ using equation [5-1]. The contributions of changes in $T_2$ and $T_2'$ to $T_2^*$ changes that occur between normal oxygenation and at death, provides some insight into the source of $T_2^*$ changes that occur during graded hypoxia.

To assess the spatial variation of changes in $T_2$ and $T_2^*$ relaxation times following death of the animal, horizontal profiles were taken at a fixed vertical position for $T_2$ and $T_2^*$ maps for each individual animal (as described in chapter 4). Upon examination of a $T_2$ profile, the region of the profile containing the gyrus was seen to have a characteristic shape (Fig [5-1]). Typically, high $T_2$ values are seen on either side of a gyrus (regions A), corresponding to the sulci, which contained CSF. Immediately next to these sulci (regions B), there is a trough in $T_2$ values, which we attributed to the thin band of cortical grey matter. Finally, at the centre of the gyrus (region C), the $T_2$ value is once again elevated and forms a peak. Region C is
identified as subcortical white matter. A similar analysis procedure was used to investigate regional changes in $T_2^*$ values.

### 5.3 Results

Table [5-1] gives $T_2$, $T_2^*$, and $T_2^\prime$ values for neonatal grey and white matter, under conditions of normal oxygenation and immediately following death of the animals ($n = 5$).

There was a significant difference ($p < 0.05$) in $T_2$ values between white and grey matter regions at normal oxygenation. The $T_2$ value of white matter was $67 \pm 6$ ms, and for grey matter $T_2$ was found to be $57 \pm 6$ ms. This is consistent with normal grey and white matter $T_2$ values previously reported. Immediately following death of the animal, there was a significant ($p < 0.05$) change from normal in the $T_2$ value of white matter regions, which fell to $55 \pm 3$ ms. However, there was no significant change, at the $p < 0.05$ level, in the $T_2$ values of grey matter following death. Additionally, upon death, there was no longer a significant difference between white and grey matter $T_2$ values.

At baseline the $T_2$ and $T_2^*$ values for subcortical white matter were found to be significantly different ($p < 0.05$). This suggests the presence of $T_2^\prime$ relaxation processes in white matter regions under non-hypoxic conditions. However, there was no significant difference between $T_2$ and $T_2^*$ values of grey matter regions at baseline, suggesting a minimal $T_2^\prime$ relaxation component. Following death there was a significant change in $T_2^*$ values in both grey ($p < 0.05$) and also white matter regions ($p < 0.05$), indicating a change in the $T_2^\prime$ relaxation component upon death in both tissues. In addition a significant ($p < 0.05$) change in the $T_2$ value of white matter was observed, whilst the $T_2$ of grey matter regions showed minimal changes in $T_2$.

$R_2$, $R_2^*$ and $R_2^\prime$ were calculated using equation [5-2] and are presented in table [5-2]. As expected from relaxation time data, $R_2$ changes were observed upon death for
white matter regions (p < 0.05), but not for grey matter. $R_2'$ relaxation rate was significantly different between white matter and grey matter regions (p < 0.05) at baseline and following death of the animals (p < 0.05). However, although a significant change in $R_2'$ relaxation rate was observed following death in white matter regions, changes in $R_2'$ were not found to be significant in grey matter at the p<0.05 level. The table again suggests the presence of $R_2'$ relaxation mechanisms in white matter under non hypoxic conditions.

Fig [5-2] shows $T_2^*$-weighted (TE = 25ms) images acquired during graded hypoxia for a single animal.

Fig [5-3] shows a plot of absolute [dHb] verses global $R_2^*$ values obtained during graded hypoxia (n = 7). As [dHb] rises, there is a concurrent increase in $R_2^*$ values. Linear regression gives a strong correlation between [dHb] and $R_2^*$ (r = 0.95). Linear regressions for the seven individual animals indicated improved correlation coefficients (0.96 < r < 0.99). The linear correlation is given by the following equation:

$$R_2^* = m[dHb] + c$$  \[5-3\]

where m = 0.466, and is the gradient of the regression line in Fig. [5-3], and c = 14.74, and is the y-intercept of the regression line in Fig. [5-3].

Fig. [5-4] shows the equivalent data for a single animal. The correlation is greatly improved (r=0.99) by the removal of the variability of individual animal responses to hypoxia. The slightly decreased correlation between [dHb] and $R_2^*$ in the combined data set may be a consequence of differences in $T_2$ values between animals. These differences could lead to slight variations in the y-intercept of regression lines for individual animals. When plotted together as in fig [5-3], these differences could lead to a less significant correlation.

For validation of the correlation between $R_2^*$ and [dHb], average global $R_2^*$, $R_2$ and $R_2'$ values were calculated and are listed in table [5-3]. There was a small but
significant change in global $R_2$ values between non-hypoxic conditions and death ($p < 0.05$). However, there were larger changes following death, in global $R_2^*$ values ($p < 0.05$) and global $R_2'$ values ($p < 0.05$). Global $R_2^*$ under nonhypoxic conditions was found to be $20 \pm 3 \text{ s}^{-1}$, and increased to $31 \pm 6 \text{ s}^{-1}$ following death of the animals. Using equation [5-3] with values for $m$ and $c$ as determined from Fig. [5-3], the changes in global $R_2^*$ described above, correspond to a calculated average ($n = 5$) global change in $[dHb]$ from $11 \pm 7 \mu\text{M}$ at baseline, to $34 \pm 14 \mu\text{M}$ immediately following death. This is in extremely close agreement with NIRS results which gave values of $12 \pm 3 \mu\text{M}$ for average global $[dHb]$ at baseline, and $37 \pm 25 \mu\text{M}$ for average global $[dHb]$ following death of the animals ($n = 5$). There was found to be no significant difference between MRI and NIRS measurements of $[dHb]$ both at baseline or following death of the animal. Individual baseline and death $R_2^*$ values are indicated in fig [5-5], plotted around the same regression line as in fig [5-3]. Baseline values are seen to be clustered together, however, the values at death reflect the myriad of physiological changes that occur in each individual animal. Yet, these values still lie along the regression line as determined in fig [5-3]. This indicates the relative importance of absolute $[dHb]$ in determining the $T_2^*$ weighted signal. Fig [5-5] also shows that upon death the average $[dHb]$ is less than that observed when the animal had reached the lowest deoxygenation step in the graded hypoxia experiment. The reason for the reduced $[dHb]$ concentration at death compared with severe anoxic conditions is because the animals were placed in a prone orientation within the stereotactic holder. Upon death NIRS measurements showed a decrease in CBV as blood drained to the base of the brain. Hence, the $[dHb]$ upon death was found to be lower than $[dHb]$ values obtained at the lowest FiO$_2$ steps.

5.4 Discussion

In this study, we have investigated the $T_2^*$ signal behaviour in response to increases in $[dHb]$. The effect of changes in CBV during our study was assumed to be small. Although there was an observed increase in CBV during the course of the experiment, the extent of changes in $[dHb]$ were on average, more than 12 times the
magnitude of CBV changes. Moreover, we believe that changes in CBV do not have an independent effect on the $T_2^*$ weighted signal intensity. The effects of CBV changes on $T_2^*$-weighted signal intensity are believed to be mediated through changes in the absolute [dHb] concentration within a particular pixel.

Unfortunately, it was not possible to investigate the behaviour or $T_2'$ with [dHb], as time constraints did not allow the measurement of $T_2$ during graded hypoxia. Acquisition of the four individual $T_2^*$ images, needed to produce a map, took approximately 10 minutes. At the lowest FiO$_2$ steps, it was not possible to maintain the animal for a further 10 minutes at each step, in order to acquire four more $T_2$ weighted images needed to produce an adequate $T_2$ map.

Recent publications have demonstrated a correlation between $\Delta R_{2^*}$ and changes in dHb $^2$. Results presented in this chapter demonstrate a quantitative linear relationship between $R_{2^*}$ and absolute [dHb]. Additionally, as absolute $T_2$ and $T_2^*$ values at normal oxygenation and immediately following death were measured, it was possible to infer the mechanism of the $T_2^*$ changes that were observed during graded hypoxia. The results suggest a difference between cortical grey and subcortical white matter regions, in the contribution of $T_2'$ relaxation to $T_2^*$-weighted signal changes during hypoxia. Rapid $T_2$ changes were observed in white matter upon death, however, $T_2$ values of grey matter remained unchanged. Changes in $T_2^*$ of white matter regions during hypoxia could be caused by changes in both $T_2$ and $T_2'$, whereas changes in grey matter may predominantly be caused by $T_2'$ changes. This difference in the mechanism of $T_2^*$ signal change between white and grey matter during deoxygenation may reflect differences in cellular / vascular structure between the two tissue types. At baseline we also measured a larger $R_2$ relaxation rate in grey matter than in white matter regions. However, $R_2'$ relaxation was higher in white matter than grey matter.

Studies have shown that cortical grey matter has approximately twice the capillary density of white matter regions $^9$. We suggest that it is the difference in capillary density between white and grey matter regions that may produce some of the observed differences of $T_2^*$ signal behaviour upon deoxygenation. Susceptibility
contrast based relaxation behavior has previously been modelled by Kennan et al. Both T2 and T2* signals were shown to be affected by diffusive processes. The model divided spin dephasing into three regimes. The slow regime, where diffusional displacement is small compared to spatial magnetic field variation. The intermediate regime, where diffusional displacement is the same order as spatial magnetic field variations; and the fast regime, where diffusional displacement is large with respect to spatial magnetic field variations. We believe that in brain tissue, the relative magnitude of these three regimes depends upon the absolute amount of susceptibility based contrast agent ([dHb]) within capillaries, the average diffusion of perivascular spins, and also the capillary density. Furthermore, we believe this dependency to be exaggerated at high B0 fields, because the susceptibility difference of [dHb] has a larger absolute effect. We suggest that the densely packed capillary structure of grey matter investigated at 7 T biases signal behaviour toward the fast regime, where magnetic field variations over space occur rapidly. On the other hand, it may be that the slow or intermediate regime dominates in white matter at 7 T, which has half the capillary density and, therefore, slower magnetic field variations over space.

Kennan et al also indicated the importance of water diffusion upon T2 signal behaviour. There is only a small difference of the directionally averaged apparent diffusion coefficient (ADCav) between grey and white matter (0.82 x 10^-9 m^2/s and 0.94 x 10^-9 m^2/s respectively). Diffusion of water molecules in the presence of static magnetic field susceptibility gradients was shown to cause an elevated R2 relaxation rate, where diffusive losses were quantified using R2^diff (the diffusion dependent R2 relaxation rate component). R2^diff was predicted to increase with increasing capillary density. A higher capillary density may lead to a greater number of spins in close proximity to capillaries. These spins then experience a greater range of field inhomogeneities as they diffuse through static field gradients. This effect may be partly responsible for a larger recorded R2 relaxation contribution for grey matter regions under normal oxygenation conditions.

If we assume the fast regime for grey matter at normal oxygenation, then diffusion processes act to decrease R2^*. Relaxation through motional averaging of the
fields seen by the water protons leads to line narrowing. The results presented in this chapter suggest that at normal oxygenation, and measured at high field (7 T), susceptibility based signal losses in grey matter do not contribute significantly to $T_2'$ relaxation. This finding is consistent with predictions made by Kennan et al for dephasing in the fast regime. However, in the slow or intermediate regime presumed to occur in white matter, static line broadening effects are still present, and hence, there is a significant $R_2'$ relaxation rate contribution to $R_2^\ast$.

$R_2$ and $R_2^\ast$ relaxation rate changes between non hypoxic conditions, and immediately following death of the animal, have been reported in both grey and white matter regions. There was found to be no change in $R_2$ relaxation rate in grey matter, an increase in the $R_2$ relaxation rate observed in white matter, and an increase in both grey and white matter $R_2'$ relaxation rates immediately following death. A number of physiological changes occur upon death of the animal. There was found to be up to 140% increase in absolute [dHb], associated with complete deoxygenation of the blood. There was also found to be up to a 40% decrease in CBV, which suggests drainage of blood away from cortical areas upon death of the animal. The drop in the CBV of cortical regions may have been accomplished either by a decrease in vessel radius or by a decrease in total number of patent vessels. After death, the blood is assumed to be completely deoxygenated, and the increase in [dHb] already takes account of a 40% decrease in CBV. However, the correct prediction of [dHb] on death using equation [5-3] and fig [5-2] confirms our assumption that CBV changes during hypoxia have a minimal effect compared with the larger variations in [dHb]. Additionally, we have previously reported changes in ADC$_{av}$ of water molecules upon hypoxia. Therefore, we assume that changes in ADC$_{av}$ of the order of 50% occur immediately following the death of the animal, as observed by others. The relative contribution of these factors upon $R_2$ and $R_2'$ relaxation rate may be dependent upon the dominant spin dephasing regime present in an individual tissue, as described by Kennan et al. In the case of ADC$_{av}$ changes, the sign of the change may also be dependent upon the dephasing regime within the tissue. The relative contribution of each physiological change upon $R_2$ and $R_2'$ relaxation rates should vary between tissues of different vascular and cellular structure.
Hence, we believe that the differences in the response of grey and white matter $R_2$ and $R_2'$ changes upon death reflect these fundamental structural differences.

In conclusion, we have demonstrated the ability of MRI to produce $R_2^*$ values of the neonatal piglet brain which under controlled conditions, enable [dHb] to be assessed. Unlike NIRS, which gives a global measure of [dHb], quantitation of $R_2^*$ can produce high resolution relaxation rate maps related to [dHb] distribution in the brain.

In the future, we hope to use $T_2'$ measurements to quantitatively map regional blood deoxygenation. Although a multitude of relaxation mechanisms contribute to $T_2$, the value of $T_2'$ is in the absence of these potentially confusing influences caused by biological changes. Hence, changes in $T_2'$ are likely to be even more strongly related to variations in [dHb].
References


<table>
<thead>
<tr>
<th></th>
<th>$T_2$ (ms)</th>
<th>$T_2^*$ (ms)</th>
<th>$T_2'$ (ms)</th>
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<tr>
<td>Grey Matter (Normal)</td>
<td>57 ± 6.5</td>
<td>54 ± 2.9</td>
<td>1450 ± 1530</td>
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<td>Grey Matter (Dead)</td>
<td>54 ± 5.4</td>
<td>42 ± 8.2</td>
<td>240 ± 120</td>
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<td>White Matter (Normal)</td>
<td>67 ± 6.2</td>
<td>47 ± 5.1</td>
<td>190 ± 90</td>
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<td>White Matter (Dead)</td>
<td>55 ± 3.1</td>
<td>30 ± 7.7</td>
<td>79 ± 40</td>
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</table>

Table [5-1]

The table shows average ($n = 5$) grey and white matter measured $T_2$, $T_2^*$ and derived $T_2'$ values at normal oxygenation and immediately following death. Reported values are ± 1 SD.

<table>
<thead>
<tr>
<th></th>
<th>$R_2$ (s$^{-1}$)</th>
<th>$R_2^*$ (s$^{-1}$)</th>
<th>$R_2'$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grey Matter (Normal)</td>
<td>17.6 ± 1.9</td>
<td>18.6 ± 1.0</td>
<td>1.6 ± 1.6</td>
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<td>Grey Matter (Dead)</td>
<td>18.7 ± 1.9</td>
<td>24.4 ± 4.8</td>
<td>6.2 ± 2.4</td>
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<tr>
<td>White Matter (Normal)</td>
<td>15.1 ± 1.4</td>
<td>21.3 ± 2.3</td>
<td>5.8 ± 4.2</td>
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<tr>
<td>White Matter (Dead)</td>
<td>18.2 ± 1.0</td>
<td>34.8 ± 8.4</td>
<td>16.6 ± 7.8</td>
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</tbody>
</table>

Table [5-2]

The table shows average ($n = 5$) grey and white matter measured $R_2$, $R_2^*$ and derived $R_2'$ values at normal oxygenation and immediately following death. Reported values are ± 1 SD.

<table>
<thead>
<tr>
<th></th>
<th>Global $R_2$ (s$^{-1}$)</th>
<th>Global $R_2^*$ (s$^{-1}$)</th>
<th>Global $R_2'$ (s$^{-1}$)</th>
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</thead>
<tbody>
<tr>
<td>Normal</td>
<td>15.9 ± 0.7</td>
<td>20.1 ± 3.2</td>
<td>4.2 ± 2.7</td>
</tr>
<tr>
<td>Dead</td>
<td>17.2 ± 1.3</td>
<td>30.8 ± 6.4</td>
<td>13.5 ± 5.3</td>
</tr>
</tbody>
</table>

Table [5-3]

The table shows average ($n = 5$) global measured $R_2$, $R_2^*$ and derived $R_2'$ values at normal oxygenation and immediately following death. Reported values are ± 1 SD.
Fig [5-1]  Diagram indicating the profile analysis procedure used to investigate spatial variations in $T_1^*$ and $T_2$ maps.
Fig 5-2  $T_2^*$-weighted images (TE = 25 ms, TR = 84 ms, matrix size = 256 x 128). Images were acquired following a stepwise decrease in FiO$_2$. [dHb] as measured by NIRS at each FiO$_2$ step is indicated below each image.
Fig [5-3] Graph showing the correlation between $R_2^*$ as measured by MRI and [dHb] as measured by NIRS ($n = 7$, $r = 0.95$). $R_2^* = m \times [dHb] + c$, where $m = 0.466$ and $c = 14.74$. 
Fig [5-4]  Correlation between $R_2^*$ and [dHb] (measured by NIRS) for an individual animal. The correlation ($r = 0.99$) is improved by the removal of biological variability of response to hypoxia.
Fig [5-5]  Regression line from fig [5-3], with baseline measurements and measurements following death (data was not used in fig [5-3]). Baseline measurements are grouped; measurements after death are diffuse, but lie along the trendline.
CHAPTER 6

Simultaneous $T_2$ and $T_2^*$-Weighted MRI Using a Modified Form of Stimulated Echo
6.1 Introduction

$T_2^*$-weighted MRI is proving to be a valuable tool for investigating brain function by providing BOLD image contrast that is sensitive to the oxygen content of blood. However, both $T_2$ and $T_2'$ are known to change in response to brain activation, and separation of the two effects may provide valuable additional information on cerebral haemodynamics.

Recently, studies have been performed into the feasibility of assessing brain iron concentrations using $T_2'$ quantisation. These measurements have indicated that a potential clinical application may be in the detection of Parkinson's Disease, and studies of other movement disorders. The investigation required calculation of $T_2'$ from $T_2$ and $T_2^*$ maps which were acquired in a novel and efficient multi-echo sequence.

A variation of the stimulated echo sequence can be used to exploit the existence of hitherto unexploited echoes that can provide $T_2^*$-weighted contrast from the same transverse spin magnetisation responsible for the appearance of the standard stimulated spin echo. The application of a suitable gradient allows either $T_2$-weighted or $T_2^*$-weighted echo to be chosen with both types of echo having a transverse relaxation weighting defined by timing that commences from the first $90^\circ$ radiofrequency (RF) excitation pulse of the sequence. The ability to rapidly switch between acquisition of either echo allows simultaneous $T_2$ and $T_2^*$ measurements to be performed which could be used to rapidly evaluate $T_2'$. 

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In this chapter, the origin of the modified echoes are described and preliminary results are presented. The source of the relaxation behaviour of the echoes is also discussed.

6.2 Method

Fig [6-1] shows the stimulated echo pulse sequence used to generate the modified echo series. The subscript "s" following the RF pulse excitation angle denotes a slice-selective pulse. Two sequences of three echoes arise following the application of the second and third 90° non-slice selective RF pulses (echo sequences A and B respectively). The origin of the echoes can be understood using the phase-graph formalism. Fig [6-2] shows the phase graph for signal coherences produced by a sequence similar to fig [6-1]. For simplicity of presentation, the read out gradient pulse timing was modified as shown at the top of fig [6-2]. Echo $A_1$ arises from the $F_1^*$ state following the second RF pulse, and because of phase inversion relative to the $F_1$ state, is $T_2$-weighted. Echo $A_2$ is a gradient recalled version of echo $A_1$, and thus contains a mixture of $T_2$ and $T_2^*$ contrast. Echo $A_3$ has arisen from the $F_1$ state and, thus, is $T_2^*$-weighted. If the second RF pulse had been slice-selective, the associated slice-selection gradient would have removed this second form of echo by dephasing. Prior to the third RF pulse, all transverse signal coherences are removed using gradient crushers. The third 90° RF pulse creates a further three echoes ($B_1$, $B_2$ and $B_3$) from $Z_1^*$ and $Z_1$ states (stored longitudinal magnetisation states) that were originally generated by application of the second RF pulse. Echoes $B_1$ and $B_2$ are $T_2^*$-weighted echoes relative to the timing of the first RF pulse with an additional small amount of $T_1$-relaxation that occurred during the mixing period. Echo $B_3$ is mainly $T_2$-weighted because it has arisen from the $Z_1^*$ state, with a small amount of $T_1$-weighting defined by the mixing period. Assuming perfect 90° RF pulses, and in the absence of relaxation effects, all echoes have an equal magnitude of $1/2$ relative to a perfect spin echo signal.
Whilst the phase-graph is useful for understanding the origin of these echoes, K-space provides a better way of understanding the image reflections that occur along read and phase-encoding axes. Fig [6-3] shows that, following phase and read axis encoding during the first evolution period, signal evolution has reached a point in K-space corresponding to F1 in the phase-graph formalism. Following the second RF pulse, the F1 and F1* coherences exist at diagonally opposed positions in K-space and proceed to evolve in subsequent gradients accordingly. The direction of K-space traversal determines the appearance of the image following Fourier transformation. Therefore, the image from Echo A1 is a mirror image of the image from echo A3 along both X and Y axes, whereas the image from echo A2 is a mirror image of that from echo A1 only along the Y axis.

6.3 Results

Images were obtained using a 7 Tesla / 20cm bore magnet and a Bruker Biospec spectrometer. A birdcage RF coil was used to provide a uniform field of view. Fig [6-4] shows images obtained from the A and B series of echoes from a tomato using the sequence of fig [6-1] (TR = 0.5 s, TM = 35.7 ms, TD = 6 ms, TE = 32.6 ms, slice thickness = 4 mm, 256 x 128 image matrix). In this experiment it was necessary to remove the slice selection gradients for the second and third RF pulses (fig [6-1]). As previously mentioned, this ensures that the T2*-weighted signal is not dephased by the slice selection gradients, although this would not have had an effect on the stimulated spin echo signal provided the gradient pulses were symmetric about the RF waveform. The image from the A1 echo is T2-weighted with an equivalent echo time of 32.6 ms (TE), whereas the image from the A3 echo is T2*-weighted with an equivalent gradient echo time of (TE + 2*TD=44.6 ms). The images have not been reflected to indicate that the orientation of each is as predicted by K-space. Therefore, the image produced from the A3 echo must be reflected around both horizontal and vertical axes for comparison to
the image from the A₁ echo. The image from the B₁ echo is T₂*-weighted with an
equivalent gradient echo time of (TE - 2*TD=20.6 ms), and the image from the B₃ echo
is T₂-weighted corresponding to a spin-echo time of TE.

Fig [6-5] shows T₂ and T₂*-weighted images obtained from standard spin echo
and gradient echo sequences with the same durations of transverse relaxation as for the
equivalent echoes of fig [6-4]. The images are now displayed after the necessary
reflections in order to facilitate direct comparisons. The T₂ -weighted image was
obtained with TE = 32.6 ms, and the T₂*-weighted images were obtained with gradient-
echo times of 44.6 ms and 20.6 ms respectively. It is readily apparent that the image
from each echo shows the predicted contrast behaviour. The image of echo A₁ is very
similar to the T₂-weighted image apart from a factor of two in signal intensity. Slight
differences between respective images are attributable to T₁ relaxation that occurred
during the mixing period for the images obtained from the B series of echoes.

6.4 Discussion

A modified stimulated echo sequence has been demonstrated that can
simultaneously obtain both T₂ and T₂*-weighted images from the same NMR signal.
This should enable these parameters to be separated and rapidly measured in future
studies. Since the mixing period can be kept quite short, the B series of echoes may
experience only a small amount of additional spin-lattice relaxation and could be added
to the corresponding A series of echoes to improve signal to noise (SNR) ratios. This
would provide a √2 advantage in SNR compared with the standard stimulated echo
experiment, but still give echoes which are a factor of 1/√2 less than could be obtained
using standard gradient or spin echo sequences.

In other variations of the sequence it is equally possible to reverse the order of
relaxation weighting through reversal of the read gradient waveform following either the
second or third RF pulse. The previously mentioned mirror-imaging of images about the phase encoding axis may be removed by applying the phase encoding gradient prior to echo formation in the period following either the second or third RF pulse.

A multi-slice version of the sequence can be produced by replacing the second and third RF pulses with slice-selective RF pulses. However, in order to refocus the echoes generated by \( F_1 \) and \( Z_1 \) states (gradient echoes), which have experienced the associated slice selection gradients, a refocussing gradient pulse must be applied prior to each of these echoes. If these echoes are followed by spin echoes, as in the B series of echoes of fig [6-2], this refocussing must be removed with a negative gradient pulse following the gradient echo in order to preserve spin echo coherence.

The additional echoes described in this chapter may have other uses including halving the number of gradient reversals required to generate an echo-planar image. Use of the principle in spectroscopy is limited by the inability to actually reverse chemical shift evolution. This may account for the fact that this echo has previously not been exploited. However, in J-modulated spectroscopy, it is possible to reverse spin-spin coupling evolution by inversion of the coupled nucleus suggesting possible applications in this area.
6.5 References


Fig 6-1 | Modified stimulated echo imaging experiment to include extra readout gradients following the second and third RF pulses in order to sample the A and B echoes respectively. Note that only the first 90° RF pulse is slice selective (90°). The effective echo time is TE, the mixing period is TM, and echoes are displaced relative to each other by a time TD.
Fig [6-2] Phase graph diagram of a sequence similar to fig [6-1] (gradient timing modified for simplicity of presentation). Only the \( F_i \), \( F_i^* \) states (solid lines), and \( Z_i \) and \( Z_i^* \) states (dotted lines) are shown together with the signals observed in the transverse plane \((u,v)\) when the phase graph trajectory passes through this plane. Further coherences from the \( F_i \) and \( F_i^* \) states following the second RF pulse are removed with crusher gradients.
Fig [6-3] Traversal of K-space for $F_1$ and $F_1^*$ coherences following the second RF pulse in response to the gradient waveform of fig [6-1]. Echoes $A_1$, $A_2$, and $A_3$ occur at the indicated positions in K-space.
Fig [6-4] Six images of a tomato obtained from the A and B series of echoes generated using the sequence of fig [6-1]. (TE = 32.6 ms, TM = 35.7 ms, TD = 6 ms, TR = 0.5s, slice thickness = 4 mm, image matrix = 256 x 128). Images are displayed without reflection along any of the axes.
Fig [6-5] Images obtained from echoes A, \(T_\tau\)-weighted, \(A_3\), \(T_\star\)-weighted and \(B\), \(T_\tau^*\)-weighted) compared with images obtained with equivalent \(T_\tau\) and \(T_\star^*\)-weighting using standard spin and gradient echo techniques (spin echo time of 32.6 ms, gradient echo times of 44.6 ms and 20.6 ms respectively).
CHAPTER 7

Summary and Discussion
CHAPTER 7

Summary and Discussion

7.1 Quantitative ADC and T\textsubscript{2} measurements in Hypoxia-Ischaemia

7.1.1 Overview

In chapter 4 we demonstrated a relationship between global ADC\textsubscript{av} value and [PCr]/[P\textsubscript{i}] ratio. Global ADC\textsubscript{av} was shown to follow changes in the [PCr]/[P\textsubscript{i}] ratio during secondary energy failure in the neonatal piglet model. ADC\textsubscript{av} was found to have a strong linear correlation with [PCr]/[P\textsubscript{i}] ratio (correlation coefficient = 0.95), suggesting that changes in ADC\textsubscript{av} during hypoxia-ischaemia reflect changes in oxidative phosphorylation. However, the non-linear correlation between T\textsubscript{2} values and [PCr]/[P\textsubscript{i}], suggests that the observed T\textsubscript{2} changes may not be as intrinsically linked to [PCr]/[P\textsubscript{i}] changes. For individual animals, significant changes in [PCr]/[P\textsubscript{i}] occurred following hypoxia-ischaemia before any change in T\textsubscript{2} was detected. This suggests that changes in T\textsubscript{2} reflect the end result of the disruption of brain metabolism (i.e. membrane breakdown leading to cell lysis).

Both ADC\textsubscript{av} and T\textsubscript{2} measurements were shown to provide detailed spatial information. ADC\textsubscript{av} and T\textsubscript{2} maps provided an opportunity to spatially investigate the pattern of brain injury following temporary occlusion of the carotid arteries. This could not be achieved by \textsuperscript{31}P MRS where localisation was limited to the use of a surface coil. Variations in both T\textsubscript{2} and ADC\textsubscript{av} values were found between cortical grey and subcortical white matter. Additionally, there was found to be a difference in the response of medial and lateral gyri following hypoxia-ischaemia. ADC\textsubscript{av} maps showed a darkening of lateral grey matter 10-12 hours before medial grey matter regions. It is suggested that this pattern of parasagittal injury reflects the vascular structure of the brain, whereby lateral gyri maybe dependent to a greater extent on
blood supplied by the carotid arteries than medial gyri. T_2 values also showed differences between medial and lateral gyri, showing a faster rise in T_2 in the lateral gyrus than medial gyrus.

Finally, the results indicate the potential importance of ADC_{av} as an early marker of secondary energy failure. Although, there were found to be no significant early changes in T_2 or [PCr]/[P_i] following the ischaemic episode, ADC_{av} values were found to be statistically (p < 0.05) lower 4 hours following ischaemia than baseline values. This initial decrease may prove to be an important indicator of impending SEP, providing a sensitive discriminatory tool to select neonates for treatment. In addition, the magnitude of the initial drop in ADC_{av} may, with further experimentation, prove to be an important indicator of neurological outcome.

### 7.1.2 Future Application of the Work

This work has already begun to be extended to the birth asphyxiated human infants. We have started to quantify ADC_{av} and T_2 values in a number of infants following perinatal asphyxia. Preliminary results confirm that there is a reduction in ADC_{av} and an increase in T_2, consistent with data using the piglet model, presented in Chapter 4.

ADC_{av} was measured using a spin-echo diffusion-weighted imaging sequence modified to allow collection of a 'navigator-echo', for retrospective correction of motion artefacts (FOV = 16 cm, image matrix = 128x128, slice thickness = 5 mm, TE = 120 ms, TR = 2000 ms, acquisition time/image = 2.2 min). Four gradient ‘b-factors’ (0, 400, 800 and 1200 s^2/mm^1) were employed separately along each of the x, y and z directions allowing calculation of absolute ADC values. Fig [7-1] illustrates typical ADC_{av} maps from a normal and an asphyxiated baby. At first glance, there appears to be little difference between the two. Upon comparison of regions of interest placed in the white matter of the frontal lobe, the ADC_{av} value of the control baby (1.7 x 10^{-9} m^2/s) was greater than that for the asphyxiated baby (1.4 x 10^{-9} m^2/s). Normal ADC values, obtained from 8 normal term infants, vary widely with
anatomical location (0.5 x 10^{-9} to 1.9 x 10^{-9} m^2s^{-1}). By a process of quantitation and careful regional analysis, it may become possible to distinguish between normal and asphyxiated infants using ADC_{av} values.

In order to quantify T_2, babies were studied in the first few days of life, and measurements were made using a 2.4 T Bruker Biospec system. T_2 values were measured using a multi-echo / multi-slice sequence. Five slices were obtained (FOV = 16 cm, image matrix = 256x256, slice thickness = 5mm). Due to the elevated relaxation times found in the immature brain, rather long echo and repetition times were chosen to increase accuracy (TE = 25, 125, 250 and 400 ms; TR = 4500 ms). Fig [7-2] shows typical T_2 maps for a control and a asphyxiated infant. Table 7-1 presents absolute T_2 values from periventricular, parietal, thalamus and frontal regions of interest from 7 control and 6 asphyxiated infants \(^1\). The results indicate an elevated T_2 from normal for the asphyxiated group, most prominent in the parietal regions.

Due to the global nature of the insult, cerebral damage is generally symmetric about the brain mid-line. Therefore, the quantitative approach as proposed in this thesis, of evaluating NMR parameters, has proved useful in the newborn perinatally asphyxiated human infant, where abnormalities cannot be detected by simple comparison of the right and left cerebral hemispheres. In stroke, where focal changes are present, DWI contrast is sufficient to detect the extent of cerebral injury.

Work is continuing to establish a database of values from normal and asphyxiated infants, with the belief that deviations in the values of these parameters from the normal range may be of clinical utility in the assessment of hypoxic-ischaemic injury.

Future work involving the piglet model of secondary energy failure, will concentrate upon testing a wide range of interventions for neuronal rescue. This would again employ the MRI and MRS techniques outlined in chapter 4. The use of mild hypothermia following the ischaemic episode is one such strategy being tested \(^2\). The exact mechanism behind the cerebroprotective effects of hypothermia remains
speculative. Early studies using the neonatal piglet model and [PCr]/[Pi] MRS monitoring have shown a statistically significant protective effect of hypothermia. However, further experimentation using the neonatal piglet model is necessary in order to detect any deleterious effects of hypothermic treatment.

Another possible cerebroprotective strategy studied in our animal model is the administration of MgSO4 following ischaemia. Once again the protective mechanism remains unclear. However, preliminary results acquired using the piglet model have shown that administration of MgSO4 does not seem to ameliorate ischaemic brain damage.

7.2 $R_2^*$ and Deoxyhaemoglobin

7.2.1 Overview

In chapter 5 we presented results that tested the relationship between $R_2^*$ value and absolute deoxyhaemoglobin concentration as determined by NIRS using the neonatal piglet model developed in our laboratory.

Upon deoxygenation global $R_2^*$ was found to increase linearly with absolute [dHb] ($r = 0.95$). At normal oxygenation average [dHb] was calculated to be $11 \pm 7 \mu M$ using the relationship between global $R_2^*$ and [dHb], which correlated well with NIRS measurements which gave a value of $12 \pm 3 \mu M$. Similarly upon death, the predicted [dHb] concentration was $34 \pm 14 \mu M$, and that measured by NIRS was $37 \pm 25 \mu M$.

Baseline values of global $R_2^*$ were found to be $18.6 \pm 1.0 \text{ s}^{-1}$ and $21.3 \pm 2.3 \text{ s}^{-1}$ in grey and white matter respectively. At death white matter $R_2^*$ was $34.8 \pm 8.4 \text{ s}^{-1}$ and $R_2^*$ values for grey matter were $24.4 \pm 4.8 \text{ s}^{-1}$. Differences between grey and white matter responses to hypoxia were believed to be partly explained by differences in capillary density and cellular structure between the two tissue types. Grey matter is
known to have twice the capillary density of white matter and to consist mainly of cell bodies.

Our results show that the absolute concentration of [dHb] per pixel is the most important factor in determining R₂* changes during deoxygenation. Additionally, the results indicate the possible future use of R₂* quantitation under controlled conditions to produce high resolution maps of brain deoxyhaemoglobin concentration, which are currently unattainable by NIRS.

7.2.2 Future Application of the Work

Further experimentation needs to be carried out to investigate the other possible contributants to T₂*-weighted signal behaviour. We have established that T₂* weighted signal is closely linked to the concentration of deoxyhaemoglobin. However, future investigations need to concentrate more specifically upon how T₂* is effected by increased blood volume, changes in brain oxygen extraction, capillary density and orientation, increased fractional oxygen saturation and blood flow changes.

We hope to develop and understand the technique of T₂* imaging in order to investigate further the utility of MRI measurements as early markers for secondary energy failure. T₂*-weighted imaging may provide such a marker as there are known to be changes in cerebral hemodynamics that precede SEF. As an initial step, work will involve application of the technique in the piglet model of secondary energy failure to test the efficacy of T₂* measurements as a means of monitoring the oxygen status of cerebral tissues.
7.3 Simultaneous $T_2$ and $T_2^*$-weighted MRI

7.3.1 Overview

$T_2^*$-weighted MRI has recently been used to investigate brain function using BOLD contrast \(^5\), and has also been shown to be sensitive to the concentration of brain iron in tissue \(^6\). There is much interest in removing the $T_2$ component of this signal in order to investigate whether the line broadening component, $T_2'$, is a more specific measure of cerebral tissue changes. However, quantitation of $T_2'$ requires measurement of both $T_2$ and $T_2^*$ values. A new method was demonstrated in chapter 6 which allows these NMR parameters to be simultaneously investigated within a single sequence using a hitherto unexploited form of stimulated / gradient echo.

Results presented include six images acquired using the new pulse sequence. The images are weighted by either $T_2$ or $T_2^*$ or another mixture of $T_2/T_2'$ relaxation. To validate the type of contrast within the images, we presented further images from standard spin echo and gradient echo sequences. We have also explained the origin of the echoes using phase graphs whereby we believe the $F_1$ and $F_1^*$ to be responsible for the $T_2^*$ and $T_2$ weighted images respectively. However, we also illustrated the formation of the echoes using K-space, which we believe better explains the orientation of the images as well as the evolution of signal.

7.3.2 Future Application of the Work

In the future, the new pulse sequence will be used in the piglet model of birth asphyxia to monitor changes in $T_2'$ following transient hypoxia-ischaemia. Also, the sequence may prove useful in further experiments involving measurements of $T_2'$ and absolute deoxyhaemoglobin as measured by NIRS. In this instance it would be possible to obtain $T_2'$ values at each desaturation step, which had not been possible until now due to time constraints of carrying out both $T_2$ and $T_2^*$ investigations separately.
7.4 References


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**Table [7-1]**

Neonatal T<sub>2</sub> values (in milliseconds ± 1SD). KEY: Periv- : Periventricular, -R : Right Hemisphere; -L : Left Hemisphere. ¹
Fig [7-1] Typical $\text{ADC}_n$ maps acquired from a control baby (left) and a perinatally asphyxiated infant (right). $\text{ADC}_n$ values from a region of interest in the frontal lobe are $1.7 \times 10^9$ m$^2$/s and $1.4 \times 10^9$ m$^2$/s for normal and asphyxiated infants respectively.
Fig [7-2]  Typical $T_1$ maps acquired from a control baby (left) and a birth asphyxiated infant (right).
APPENDIX A

Volume Selective 3D Imaging
Applied to $T_2^*$ Quantitation.
APPENDIX A

Volume Selective 3D Imaging
Applied to T₂* Quantitation.

A-1 Introduction

BOLD contrast relies on the dephasing of spins within a voxel as a consequence of changes in microscopic susceptibility differences. However, at higher field strengths the artefacts associated with T₂*-weighted images also become more prominent. The foundations of BOLD contrast are based on two assumptions. Firstly if large inhomogeneity gradients of similar magnitude to imaging gradients are present, they may result in geometrical image distortions. Microscopic inhomogeneity gradients are assumed to be of such an extent that they themselves do not cause geometrical image artefacts. Secondly, because macroscopic magnetic field inhomogeneities are generated at the interfaces between materials of widely differing susceptibilities (i.e. the skull and air, or due to ineffective shimming) the effect of these gradients over the dimensions of a pixel is assumed to be negligible compared to microscopic field inhomogeneities within the pixel.

With standard gradient echo FLASH imaging at high magnetic field strengths both these assumptions become more difficult to satisfy. Quantitative studies require the acquisition of a set of images with increasing echo times in order to produce T₂* maps. The extent of image artefacts are directly related to the echo time of the image. Hence, producing spatially useful quantitative T₂* maps from a series of increasingly T₂*-weighted images, as presented in chapter 5, is difficult at high field strengths.

Since localisation of signal requires the use of linear magnetic field gradients, any inhomogeneity in the static field, either due to imperfections in the magnet or intrinsic to the object being imaged, causes geometrical distortions that are constant
throughout the image series. Fluctuations in image intensity that increase with $T_2^*$ weighting on the other hand cause errors in $T_2^*$ estimation.

**A-2 Theory**

$T_2^*$-weighted images can be made less sensitive to macroscopic magnetic field inhomogeneity gradients by modification of the image acquisition parameters, i.e. using a smaller voxel size so that dephasing within a particular voxel as a result of macroscopic inhomogeneous field distributions across the voxel is reduced, thereby preserving signal. Microscopic inhomogeneities would have the same effect on $T_2^*$ weighted signal. Even with this improvement, the $T_2^*$-weighted imaging technique at high field strengths suffers from serious image artefacts. Although individual $T_2^*$ weighted images may appear artefact free after increasing the resolution, artefacts may still remain in $T_2^*$ maps because of the fluctuations in the intensity of images at different echo times.

To understand the fundamental causes of these image artefacts the effect of field susceptibilities on gradient echoes needs to be considered. A diagram of a $T_2^*$ pulse sequence is presented in chapter 3 (Fig [3-3]). The pulse sequence can be considered to be formed of three sets of orthogonal gradients (namely frequency, phase and slice select). The effect of inhomogeneities on each of these is considered in turn. Over the dimensions of an image pixel, these field inhomogeneities can be approximated by three gradient fields along the main Cartesian axes.

The phase encoding axis is insensitive to inhomogeneity induced gradients along both the frequency and phase-encoding directions. Since the acquisition time is identical for each column in K-space along the phase encoded axis, a constant dephasing is experienced for each time point along this axis. Magnitude FT data then provides an undistorted profile of the object along the phase encoded axis.
In the case of frequency encoding, inhomogeneity gradients have a greater effect on the time data. This is illustrated in fig [A-1] which shows that a field inhomogeneity gradient along the read axis causes part of the echo corresponding to the signal experiencing this extra gradient to shift within the acquisition window. However, provided the echo remains well within the acquisition window, this effect does not result in loss in the total signal. As illustrated in fig [A-1], the FT of the echo (profile along the frequency encoding axis) is distorted because the frequency of spins along the direction of the frequency encoding gradient is crucial to the correct mapping of signal. If inhomogeneity gradients alter the frequency of spins in a particular location then this directly effects the frequency encoded information. For this reason, the frequency encoding axis is also sensitive to inhomogeneity gradients along the phase-encoded axis. Note that in the case of both frequency encoding and phase encoding, there is no overall loss in signal providing the displaced part of the echo remains within the acquisition limits.

The effect of inhomogeneity gradients on slice selection is by far the most important. It is this effect which causes the majority of artefacts in T₂* weighted images. In the presence of inhomogeneity gradients along the slice select direction, signal intensity is directly affected. The effect is enhanced along the slice select direction because voxels are normally largest along this axis. Inhomogeneity gradients add or subtract from the slice select gradients causing improper refocussing of spins within the slice. Additionally, as inhomogeneity gradients act over the entire gradient echo time period, they cause an even larger effect on spins. The effect of inhomogeneity gradients on slice selection can be easily estimated. For a SINC RF pulse, the slice profile is approximately rectangular. Such a rectangular slice measured in the presence of a reversed gradient along the slice selection axis, would produce a SINC like echo signal. Normally, the slice refocussing gradient is removed when the maximum of this function is reached to provide the largest signal from the refocussed slice. Unfortunately, inhomogeneity gradients along this axis cannot be removed, and lead to an additional dephasing along the slice axis during the echo delay time. As a function of echo time, the signal intensity will oscillate giving severe intensity variations in images that can no longer be fit to an exponential.
There are two possible approaches to overcome the effect of inhomogeneity
gradients on slice selection. Either to produce multiple images with an incremented
slice refocussing lobe or to use a 3D imaging technique.

**A-3 Method**

The pulse sequence diagram of the volume selective 3D imaging sequence
used is presented in fig [A-2]. Following slice selection an additional phase encoding (VPE) gradient is applied along the volume selection axis. This gradient is
decremented after acquisition of each image. This decrement is adjusted to be equal
the decrement in the standard phase encoding gradient, giving an equal resolution in
both phase encoding directions.

As the sample is usually larger than the FOV along the volume select
direction, volume selection gradients are needed. The excited volume should be
encompassed within the FOV in the volume select direction.

A 3D FT applied to the acquired data then produces a set of images along the
volume select direction with a slice thickness equal to the inplane resolution. The
images may then be co-added to increase signal to noise ratio and produce a thicker
slice. The effect of phase encoding in the 3rd dimension is in effect to sample more
parts of the SINC like echo described in section A-3. Normally, the slice refocussing
gradient is removed when the maximum of this function is reached. In the presence of
macroscopic magnetic field inhomogeneities, the spins continue to dephase after the
removal of the refocussing gradient. Signal is therefore moved away from the
maximum of the SINC like function and is no longer sampled. By phase encoding
after exciting in the volume selection direction, this displaced signal is once again
sampled. Typically between 16 and 32 refocussing steps are necessary for adequate
image correction. This range of VPE steps more than covers all possible refocussing
errors and all signal is fully recovered along the 3rd axis.
The volume selective 3D sequence was tested on the newborn piglet model described in chapter 4. The animal was inserted into a purpose built pod and the head clamped with a stereotactic holder. A 6.5 cm diameter surface coil was used as a transmitter and receiver. The pod was inserted into the bore of a 7 T / 20 cm bore magnet. Shimming was performed until a linewidth of less than 40 Hz was achieved. Four increasingly T2*-weighted images were acquired using a standard FLASH sequence (TE = 6, 15, 25 and 34 ms, TR = 84 ms, FOV = 5 cm, image matrix = 128 x 128, slice thickness = 2 mm, ). The volume selective 3D sequence was used to acquire 16 images at each echo time (TE = 6, 15, 25 and 34 ms, TR = 126 ms, FOV in the frequency encode direction = 5 cm, image matrix = 128x128x16, FOV in the volume select direction = 6.25 mm, excited volume thickness = 2 mm). For comparison, images acquired using the volume selective 3D sequence were co-added to give a slice thickness of the final imaging slice which was equal to that obtained using standard FLASH imaging.

The effectiveness of the volume selective 3D sequence in reducing image signal intensity artefacts was also investigated in a phantom containing microspheres suspended in a gel matrix. 3 ml cylindrical gel phantoms were produced with various concentrations of microspheres (0 to 8 % by relative concentration). The concentration of gelatine was chosen such that the setting time was sufficiently short to prevent microspheres settling within the phantom. The phantoms were then imaged using the slice selective 3D sequence with 32 VPE steps (TE = 6, 12, 18 and 26 ms, TR = 126 ms, FOV in the frequency encode direction = 6 cm, FOV in slice selection direction = 3.75 mm, excited volume thickness = 2 mm, image matrix = 256x256). T2* relaxation times were quantified for each phantom. T2 imaging was also conducted using a standard spin echo pulse sequence with a variable echo time (TE = 35, 80, 120 and 160 ms, TR = 2080 ms, FOV = 6 cm, slice thickness = 2 mm, image matrix = 128x128). Eight images acquired at each echo time were co-added to increase signal to noise and slice thickness. T2* and T2 values were calculated using a log linear fit of signal intensity versus echo time.
A-4 Results

Fig [A-3] shows typical images acquired using the volume selective 3D sequence on the piglet model. The data shown in fig [A-3] were acquired using an echo time of 6 ms and, hence, do not show much T₂*-weighted contrast. In total, sixteen VPE step slices were acquired with a resolution along the third axis of 0.39 mm. As only a 2 mm thickness of tissue was excited by the slice selective pulse, approximately 5 out of the 16 slices contain images of the brain. Some low intensity ghost images can be seen in the slices surrounding the five image slices. This is caused by the limited number of VPE steps, which results in ringing when the Fourier transform is applied in the third dimension. The five slices containing the most intense images were added together to give a combined image with a slice thickness of approximately 2 mm. These images could then directly be compared to those acquired using the FLASH technique as shown in fig [A-4]

The FLASH image shows several types of distortion. Firstly, incomplete refocussing leads to a loss of signal intensity with increasing echo time as seen in the upper left portion of the brain. This results in an under-estimate of T₂* values from this region. The second, and more striking artefact, is a band of increased signal intensity which appears with increasing echo time. During our experiments this artefact occurred periodically and caused an over-estimate of T₂*. Although we cannot confirm the origin of this signal unambiguously, we believe that this intense signal arose from outside the imaging slice through an uncrushed coherence. This coherence was crushed at short echo times by imaging gradients, but rephases in the presence of field inhomogeneities acting over the protracted echo period. The nature of field inhomogeneities in the extended sample would make the appearance of this artefact a matter of chance.

The 3D sequence corrects for incomplete refocussing along the slice selection axis by using phase encoding. In addition to avoiding this problem with inhomogeneity gradients parallel to the slice selection axis, the difference in pulse
sequence timing and gradients seemed to make $T_2^*$-weighted imaging less prone to these hyper-intense artefacts. The $T_2^*$ maps obtained in this example confirm that, in at least this case, both forms of artefact have been improved leading to better $T_2^*$ estimates.

Fig [A-5] shows phantom data with relaxation rate plotted against the relative concentration of microspheres added to the gel matrix. High macroscopic susceptibility gradients usually make it impossible for accurate determination of $R_2^*$. However, using the 3D sequence, it was possible to study changes in $R_2^*$ relaxation rate with relative concentration of microspheres. In combination with $R_2$ relaxation rate, this allows $R_2'$ to be determined. $R_2$ relaxation rate increases linearly with the concentration of microspheres. However, $R_2^*$ relaxation rate increases at an exponential rate with the relative concentration of microspheres. $R_2'$ relaxation rate shows a similar trend.

A-5 Conclusion

The results demonstrate the effectiveness of volume selective 3D imaging in reducing artefacts in $T_2^*$ images, making possible a more accurate quantitation of $T_2^*$. Animal results showed that using 16 phase encoding steps in the slice select direction resulted in more uniform and structurally accurate $T_2^*$ maps. However, in severe cases signal is still lost due to the slice select effect discussed in section A-2. This occurs at long echo times and in tissue close to interfaces between the skull and the air. The effect of large inhomogeneity gradients generated at these interfaces increases with increasing echo time as the susceptibility gradient is present throughout the echo time. Signal at long echo times is then moved out of the range that can be corrected by 16 VPE steps. The sequence can be made more tolerant to such signal losses by increasing the number of phase encoding steps in the slice select direction.

The ideal solution for improving $T_2^*$ imaging is to remove the slice selection process altogether, using a purely 3D phase encoding based technique. The size of the
voxel generated by such a technique should be small, such that macroscopic inhomogeneity gradients across the voxel have a smaller effect. As samples are usually longer than the field of view of the coil, a volume selective pulse is required to provide an acceptable resolution for correction along the 3rd axis.

The gel phantom results demonstrate the possibility of quantifying high $R_2^*$ relaxation rate values using the 3D sequence. Upon increasing the concentration of microspheres, there was a small increase in $R_2$ and a much larger increase in $R_2^*$. The increase in $R_2$ is thought to be as a result of water molecules diffusing through static field inhomogeneities, which increase with increasing microsphere concentration.

The volume selective 3D sequence is a useful method of counteracting the intensity fluctuations found in $T_2^*$ imaging. For maximum susceptibility tolerance an increased number of VPE steps need to be applied. However, this results in an increase in the time needed for image acquisition. Thus, the method is not suitable for studies in which fast changes in $T_2^*$-weighted signal intensity changes need to be monitored. The 3D sequence is ideal for the long term monitoring of $T_2^*$, for example, in studies of perinatal asphyxia outlined in chapter 4 and the determination of deoxyhaemoglobin concentration as described in chapter 5.
Fig [A-1] (a) Diagrammatic illustration of an echo acquired in the absence of magnetic field inhomogeneities. The FT of the echo results in an undistorted profile along the frequency encoding axis. (b) Diagrammatic illustration of an echo in the presence of a magnetic field inhomogeneity over part of the sample. The FT of the echo results in a misregistration of signal from the affected area. However, total signal intensity is preserved provided that the affected signal remains within the acquisition window.
Fig [A-2] Pulse sequence diagram of a volume selective $T_2^*$-weighted 3D sequence, TE is the echo time. Phase encoding is applied along the third dimension. VPE is the phase encoding gradient in the volume select direction. The VPE gradient is decremented after acquisition of each image.
Fig [A-3] Sixteen reconstructed slices acquired using the volume selective 3D imaging sequence. The sixteen slices represent a FOV in the volume selective direction of 6.25 mm. Approximately five out of the sixteen slices contain substantial signal, representing the 2 mm of tissue excited by the volume selective RF pulse. Slice thickness = 0.39 mm, TE = 6 ms, TR = 126 ms. Signal from the five images may be co-added to produce 2 mm slices for comparison with a standard FLASH image.
(a) $T_2^*$-weighted images acquired using a standard FLASH technique. Slice thickness = 2 mm.

(b) $T_2^*$ map produced from images of (a).

(c) $T_2^*$-weighted images acquired using the volume selective 3D imaging technique. Combined slice thickness = 2 mm.

(d) $T_2^*$ map produced from images of (c).

Fig. [A-4]
Fig [A-5]  Graph showing changes in relaxation rates with an increasing concentration of microspheres in a gel matrix.
APPENDIX B

Sham Operated Control:
Quantitative ADC and $T_2$
Measurements in the Piglet Model
of Secondary Energy Failure.
APPENDIX B

Sham Operated Control: Quantitative ADC and T2 Measurements in the Piglet Model of Secondary Energy Failure

B-1 Introduction

Published control data using the piglet model of secondary energy failure indicate that in the absence of a transient hypoxic-ischaemic insult, there is no significant change in \([\text{PCr}]/[\text{Pi}]\) as determined by MRS over the 48 hour time period following the insult described in section 4.2.1. In the absence of an insult data in this appendix shows that minimal changes in global \(ADC_{av}\) and global \(T_2\) values occur over this 48 hour period.

B-2 Method

The animal preparation was conducted as outlined in section 4.2.1. A tracheotomy was carried out and anaesthesia maintained as described. Umbilical arterial and venous catheters were inserted and inflatable occludes were positioned around both carotid arteries as previously described. However, the piglet was not subjected to an hypoxic-ischaemic insult.

Diffusion weighted imaging, \(T_2\)-weighted imaging and \(^{31}\text{P}\) MRS was performed for 48 hours as described in section 4.2.2. Average global ADC, \(T_2\) and \([\text{PCr}]/[\text{Pi}]\) values were calculated for tissue within the field of view of the surface coil.
**B-3 Results**

Fig [B-1] (a) shows changes in [PCr]/[Pi] from a control animal for 48 hours following a sham insult. After an initial drop following the sham insult the [PCr]/[Pi] ratio of the control animal remains relatively constant throughout the 48 hours. This is in agreement with published data. Fig [B-1] (b) shows changes in global ADC\(_{av}\) for the same control animal as fig [B-1] (a). Global ADC\(_{av}\) values also remain relatively constant over the 48 hour period. Finally, fig [B-1] (c) presents changes in global T\(_2\) in the control animal. Over the 48 hour period there is once again little change in global T\(_2\) values for the control animal.

**B-4 Discussion**

The data suggests that in the absence of an acute period of hypoxia-ischaemia there is little change in the NMR measured parameters measured in the piglet model of secondary energy failure. This is in marked contrast with changes that are observed in piglets that are subjected to a period of hypoxia-ischaemia. In this instance large significant changes are observed over the 48 hour period as presented in chapter 4. This data indicates that observed changes in NMR parameters (as presented in chapter 4) following transient hypoxia-ischaemia are not simply a consequence of the experimental procedures that the animals underwent, but are likely to be caused by the hypoxic-ischaemic period experienced by the piglets.

More control data is currently being obtained for purposes of statistical validation.
Fig [B-1]  (a) Graph showing typical changes in [PCr]/[P_i] ratio for a control animal that has not been subjected to a hypoxic-ischaemic insult. (b) Graph showing typical changes in ADC_{av} for the same animal as in (a). (c) Graph showing typical changes in T₂ for the same animal as in (a).
References for Appendicies


Errata Sheet:*

Page 6, paragraph 1:
In 1980, Hawkes et al demonstrated the first images of abnormal human pathology

Page 21, paragraph 2:
In equation [2-17] \( \omega \) refers to the frequency of rotation of the rotating frame of reference.

Page 31, paragraph 2:
These can be in rapid exchange between the acid and base states so a ......

Page 35, paragraph 1:
The Biospec (Bruker, Karlsruhe, Germany) ......

Page 49, paragraph 2:
A diffusion weighted sequence can be created by insertion of 'diffusion sensitising' gradients on both sides of a 180° pulse of a spin echo sequence, or before the second 90° and after the third 90° pulse in a stimulated echo (fig [3-3]) sequence.

Page 131, paragraph 2:
This decrement is adjusted to be equal to the decrement ......

* Corrections are indicated in bold italic print.