Measuring Blood Perfusion in the Brain using Arterial Spin Labelled MRI

Laura Michelle Parkes

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NMR Research Unit
Institute of Neurology
University College London
Queen Square
London WC1N 3BG
Abstract

Perfusion is a fundamental biological function, giving an indication of tissue metabolism, through the rate of blood supply. Changes in perfusion accompany almost all forms of brain disease giving a wide range of potential applications for perfusion imaging. Arterial spin labelled MRI, the subject of this thesis, is a good method of measuring brain perfusion.

Issues of perfusion quantification, i.e. accuracy and precision are addressed. Current models of the perfusing system assume that water is freely diffusible across the capillary wall. Several published values show that this is not true in the brain where the blood brain barrier restricts water passage. A corrected two-compartment model is presented, with simplifications for use in vivo. Simulations show that the change to perfusion estimation is large. In vivo modelling shows an improved fit for all extremes of perfusion.

Perfusion reproducibility is measured for different tissue volumes and is found to compare favourably with other perfusion techniques. A study of thirty-two normal volunteers shows that inter-subject perfusion variation is large, but perfusion within a single subject remains fairly stable over the course of a day and a week. A significant (p<0.05) negative correlation of grey matter perfusion with age is reported, giving a perfusion decrease of 0.5% per year. Female whole brain perfusion is found to be 16% higher than in males (p=0.02).

Measurements of perfusion change are made in stroke, arteriovenous malformation (AVM), motor activation and multiple sclerosis. The examples in stroke and motor activation serve as important validation of the technique and the modelling. Gross perfusion abnormalities are detected in AVM showing different perfusion characteristics in different regions of AVM. More subtle changes are found in multiple sclerosis, with significant perfusion increases in the normal appearing white matter compared to normal controls.
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List of Abbreviations

ASL - Arterial Spin Labelling.
AVM - Arteriovenous Malformation.
CASL - Continuous Arterial Spin Labelling.
EPI - Echo-planar Imaging.
EPISTAR - Echo Planar Imaging and Signal Targeting with Alternating Radio frequency.
FAIR - Flow Alternating Inversion Recovery.
fMRI - functional Magnetic Resonance Imaging.
GE - Gradient Echo.
GM - Grey Matter.
MRA - Magnetic Resonance Angiography.
MRI - Magnetic Resonance Imaging.
MS - Multiple Sclerosis.
MT - Magnetisation Transfer.
MTT - Mean Transit Time.
NMR - Nuclear Magnetic Resonance.
PASL - Pulsed Arterial Spin Labelling.
PET - Positron Emission Tomography.
PICORE - Proximal Inversion with Control for Off-Resonance Effects.
QUIPSS - Quantitative Imaging of Perfusion using a Single Subtraction.
PS - Permeability Surface area product.
rf - radio frequency.
SE - Spin Echo.
sd - standard deviation.
SPECT - Single Photon Emission Computed Tomography.
WM - White Matter.
Novel Findings of this Thesis

The following advances were made, with references to publications given overleaf.

1. A two-compartment model for perfusion quantification is introduced with simplifications for application in vivo [1,6,8,9,10].

2. The reproducibility of the perfusion measurement technique of continuous arterial spin labelling compares favourably to other perfusion measurement techniques [2].

3. Perfusion within a subject remains relatively stable over the course of a day and a week [7].

4. There is a significant (p<0.05) negative correlation of grey matter perfusion with age, giving a perfusion decrease of 0.5% per year. Female whole brain perfusion is 16% higher than in males (p=0.02) [2].

5. There is a significant increase in the white matter perfusion in relapsing and remitting (p=0.005) and secondary progressive (p=0.04) subgroups of multiple sclerosis compared to normal controls [4,5].

6. Arterial spin labelling is an interesting technique for the measurement of perfusion in arteriovenous malformations [11,12].

7. Local spatial correlations in brain perfusion are fractal [3].

8. Techniques for perfusion measurements in the brain are reviewed [13].
Publications Arising from this Thesis


Chapter 1

Introduction

1.1 A history of human blood flow measurements

1.1.1 The macrovasculature

Blood circulation has been a subject of fascination from the times of Hippocrates to the present day. Interest and understanding has gone hand in hand with advances in microscopy and medicine, with early work concentrating on the heart and lungs. Without the need for special equipment it was possible to feel the heart beat and the lungs fill with air and to examine these organs in dissection.

The brain however was more of a mystery being encased in the skull during life and holding little of structural interest in dissection. The superficial vessels of the brain were described in Hippocratic times but the interior of the brain was thought to be bloodless [100]. Galen (A.D. 129 - 199) collated the ideas of the Greeks and recognised that the brain required some substrate to create ‘animal spirit’ to drive cerebral function.

During the Renaissance little progress was made until Vesalius who, through careful dissection, gave the most complete description of the vessels of the brain for many years to come. Book III of De humani corporis fabrica of 1543 contains a detailed drawing of these vessels, reproduced in figure 1.1 [204]. When compared to a modern day diagram of the cerebral vasculature (figure 1.2) [136] many errors can be seen for example it is now known that there is no connection between the carotid artery and the transverse sinus. Later came Thomas Willis whose work Cerebri anatome nervorumque in 1676 gave an extensive description of the anastomotic circle of arteries at the base of the
Figure 1.1: *The blood vessels of the head from Book III of De humani corporis fabrica* Vesalius, 1543.

Figure 1.2: *The arteries and veins of the head from Faber's Anatomical Atlas, 1964.*

brain, the circle of Willis.
1.1.2 The microvasculature

Although knowledge of the gross vascular anatomy was quite detailed at this time very little was understood about brain metabolism and the capillary system. This changed with the advent of microscopy and Malpighi’s first view of the lung capillaries in 1661:

\[ \text{Here it was clear to sense that the blood flows away through tortuous vessels, that it is not poured into spaces but always works through tubules, and dispersed by the multiplex winding of the vessels.} \]

In 1674 with higher magnifying power van Leeuwenhoek saw that:
\[ \text{arteries and veins are one and the same continued blood vessels.} \]

This opened up ideas and research into the capillary system of the brain. Many discrepancies in the observation and interpretation arose from the lack of any clear definition of the term capillary. This problem remains today as can be seen from a discussion at a conference in 1988:

\[ \text{Mchedlishvili - I would say that it is just those arterial branches of approximately 100 \( \mu \text{m} \) in diameter that should be considered as the boundary of the microvascular bed of the cerebral cortex.} \]

\[ \text{Kislyakov - In my view the criterion should be based primarily on the functional signif- icance of the vessels, i.e., on the transport of substances to the tissue microregions.} \]

\[ \text{Schmidt-Schönbein - In macrovessels the blood flows as a fluid with definable properties. In the smaller vessels the two-phase nature of the blood becomes dominant. We are thus justified in talking about a rheological system in the microcirculation which is really different from that in the macrocirculation.} \]

Perhaps one of the most succinct definitions came from Marshall Hall in 1831:

\[ \text{The minute vessels may be considered as arterial, as long as they continue to divide and subdivide into smaller and smaller branches. The minute veins are those vessels which gradually enlarge from the successive addition of smaller roots. The true capillary vessels are obviously distinct from each of these. They do not become smaller by subdivision, nor by conjunction; but they are characterised by continual and successive } \]
union and division, or anastomoses, whilst they retain a nearly uniform diameter [81].

Figure 1.3: A schematic diagram showing how some of the properties of blood vessels change on moving through the vasculature from artery to vein.

It emerges that a continuum of vessel sizes is a more realistic view than trying to define a division between macro and microvasculature. Properties such as diameter, blood velocity, wall permeability and oxygen concentration all change continuously on moving through the vasculature from artery through capillary to vein. Figure 1.3 shows schematically how some of these properties are likely to change. Any categorisation into arteries, arterioles and capillaries are arbitrary cut-off points. In my opinion, a continuum view is more useful than categorising into capillaries, arterioles and arteries.

1.1.3 The Fick principle

Research up until the late 19th century involved detailed observation and description but few quantitative measurements. With improved instrumentation and increasing intrigue into brain metabolism and blood flow regulation the first human measurements were made. A significant advance came in 1927 through the development of a technique for safe sampling of human venous and arterial blood from the internal carotid artery and the internal jugular vein [145]. This allowed Lennox and Gibbs to measure arteriovenous oxygen change and then use the Fick principle [54] to estimate cerebral blood flow [118]. The Fick principle states that the quantity of oxygen taken up by the brain, \( Q \) (mol) per unit time, \( t \) (min), is equal to the product of blood flow through the
brain, $F$ (ml/min), and the difference in arterial ($C_a$) and venous ($C_v$) (mol/ml) oxygen concentration, or:

$$\frac{dQ}{dt} = F(C_a - C_v) \quad (1.1)$$

This is based on a much older idea - simple mass conservation. Lennox and Gibbs assumed that the rate of oxygen uptake, $dQ/dt$, was constant and hence that blood flow was proportional to the inverse of the arteriovenous concentration difference. However, as Kety realised:

"Consumption [of oxygen] cannot independently be measured or even assumed to be constant since it would be expected to vary with activity and disease" [99].

In 1944 Schmidt gave a dismal view of the state of cerebral blood flow measurements:

"It is unfortunate indeed that now, when data concerning the behaviour of the human cerebral circulation are urgently desired, the only thing that can be said is that a considerable amount of fundamental research will have to be done before such data become available. Yet in the past the first step towards advances in clinical experimentation has often been dissatisfaction with the methods previously utilized, and it is to be hoped that history will repeat itself here [177]."

This spurred Kety on [100] to make the first accurate measurements of cerebral blood flow in man later that year. Measurements were based on the Fick principle but with the use of inhaled Nitrous Oxide, $N_2O$ as an inert tracer that would be unlikely to be affected by functional state or disease. The other benefit of $N_2O$ is that it is freely diffusible through the capillary wall and brain such that equilibrium between $N_2O$ concentrations in the brain and venous blood are quickly reached. This removes the need for a measurement of tissue uptake of $N_2O$, $Q$, since it is proportional to the venous blood concentration ($C_v$) through the proportionality constant $\lambda$, multiplied by the mass of the brain $W$, i.e. $Q=W\lambda C_v$. Kety used this fact to convert the Fick principle to:

$$\frac{d(W\lambda C_v)}{dt} = F(C_a - C_v) \quad (1.2)$$

such that

$$\frac{F}{W} = \frac{\lambda C_v(T)}{\int_0^t (C_a - C_v) dt} \quad (1.3)$$
Knowledge of the proportionality constant, $\lambda$ [98] and measurement curves for arterial and venous $\text{N}_2\text{O}$ concentration with time, $C_a(t)$, $C_v(t)$ until equilibrium ($t=T$) were all that was required for a blood flow measurement. Figure 1.4 shows the experimental set-up and the original measurements [101]. Cerebral blood perfusion is defined as $f=F/W$ and was measured as 57.5 ml 100 g$^{-1}$ min$^{-1}$ [97].

![Experimental set-up](image)

Figure 1.4: The original experimental set-up and results from the first human cerebral blood flow measurements by Kety and Schmidt, 1944. $A$ is the arterial concentration curve and $V$ the venous curve. Perfusion is proportional to the inverse of the area between these two curves.

We can see that the units for blood perfusion are easily understood using this technique as the volume of blood passing through the brain normalised to the mass of the brain. The term blood ‘perfusion’ is used to replace blood ‘flow’ in order to avoid confusion with blood velocity. Perfusion is a measure of blood supply to the tissue.

The use of $\text{N}_2\text{O}$ as a tracer naturally lead on to the use of other tracers, in particular the use of radioactive inert gases that would permit regional measurements of cerebral blood perfusion. The need for regional measurements increased with advances...
in surgery, therapeutic drugs and understanding of brain function. In turn our ideas about the cerebral circulation and function have been shaped by the evolving technology.

1.2 Modern day human blood perfusion measurements

This leads us to methods currently in use; all based on the Kety model of tracer uptake. Current methods involve either the use of radionuclides as tracers with radiation detectors or magnetic tracers with Magnetic Resonance Imaging (MRI). The use of detectors that can measure tracer concentration in different brain areas, rather than only sampling blood concentration as with N₂O, allows regional perfusion measurements. The radionuclide methods can be distinguished on the basis of the physical properties of the radionuclides used. Low-energy photon-emitting radionuclides are the tracers used in single photon emission tomography (SPECT) and positron-emitting radionuclides are used in positron emission tomography (PET).

1.2.1 SPECT

1.2.1.1 Xenon clearance

Radiolabelled Xenon ^133Xe can be used in place of N₂O. Regional concentration of Xenon can be measured using arrays of gamma detectors. A solution to equation 1.1 is used with the venous concentration \( C_v \) expressed in terms of the tissue concentration \( C \), \( C_v = C/\lambda \). Measurements of the arterial concentration time curve \( C_a(t) \), and the tissue concentration clearance curve \( C(t) \) can be used to fit the model curve for perfusion. The main errors in the measurement arise due to recirculation of the indicator and poor accuracy of the arterial time curve. This technique is unreliable for low perfusion measurements [71]. It can however be used at the patient's bedside and is relatively cheap.

1.2.1.2 \(^{99m}\)Tc-HCMPAO and IMP

The compounds \(^{99m}\)Tc]hexamethylpropyleneamineoxime \((^{99m}\)Tc-HCMPAO) and isopropyl[^123I]-p-iodoamphetamine (IMP) are lipophilic and quickly pass through the
blood brain barrier. Once in the brain they go through a metabolic change into hydrophilic compounds that cannot cross back into the blood. Thus the venous tracer concentration is zero and equation 1.1 with $C_v = 0$ and $Q(t) = C(t)W$ reduces to:

$$f = \frac{C(T)}{\int_0^T C_a(t)dt}$$

(1.4)

Perfusion is directly proportional to the regional concentration of tracer $C(T)$ which is again measured with gamma detectors. These compounds produce higher energy photons than $^{133}$Xe with less scatter, allowing a higher resolution image. Advances in detection through the use of multiple, rotating gamma cameras has improved the spatial resolution to about 2cm³ [71].

1.2.2 PET

The radionuclides used in PET emit positrons, which on annihilation produce two high energy gamma rays travelling in opposite directions. This allows the use of coincident gamma ray detection which can pinpoint the position of the radionuclides providing better resolution than SPECT. The usual tracer is $^{15}$O which is inhaled as $^{15}$O$_2$ and transferred to $^{15}$H$_2$O in the lungs. Perfusion is underestimated at high flows due to limited water diffusion across the capillary wall. This is reduced with the use of more diffusible tracers such as butanol [86]. The great advantage of PET is the possibility of simultaneous measurements of oxygen and glucose metabolism and perfusion through the use of different tracers. Main disadvantages are the need for an on-site cyclotron to synthesize the short-lived radionuclides, and the relatively high radiation doses which do not permit repeat measurements.

1.2.3 MRI

One great attraction of perfusion imaging using MRI is the ability to register the perfusion images with high resolution structural images. Rather than detecting radiation emitted by a tracer MRI detects the magnetic field induced by a magnetic tracer. This will be explained in detail in the next chapter. There are two principle methods of perfusion imaging using MRI - one using an exogenous tracer, coined ‘bolus tracking’ and the other using magnetically labelled blood water as an endogenous tracer, termed Arterial Spin Labelling or ASL.
1.2.3.1 Bolus tracking

Bolus tracking involves the intravenous injection of a paramagnetic contrast agent, usually gadolinium-based. As the tracer passes through the cerebral vasculature the magnetic signal is reduced by the order of 20%. This reduction can be linked to the concentration of the tracer in the tissue voxel. The tracer remains intravascular and so the measurement includes signal from all blood vessels, causing uncertainty over whether this technique is measuring true capillary perfusion. Confounding problems include: an accurate measure of the arterial concentration; delay and dispersion of the bolus; an accurate relationship between the concentration of the tracer and the signal change; mathematical difficulties in deconvolving the arterial and tissue signal; leakage of the tracer through the blood brain barrier. All these problems make accurate quantification uncertain. Due to the mild toxicity of the tracer frequently repeated measurements are not possible.

1.2.3.2 Arterial spin labelling

Rather than injecting a tracer, Arterial Spin Labelling or ASL uses blood water itself as an endogenous tracer. This is less expensive and more pleasant for the patient but most importantly permits frequent rescanning. Serial measurements could be useful in a number of applications for example following perfusion changes after stroke or drug treatment, during sleep or brain activation. Flowing blood is magnetically ‘labelled’ providing the contrast for the perfusion measurement.

The technique offers other advantages over the older techniques. It provides a closer measure of true capillary perfusion than the bolus tracking method with methods of sensitising the measurement to different vessel sizes. Quantification is simplified due to the known relationship between the signal and the concentration of labelled molecules unlike bolus tracking where this relationship is dependent on vessel size and architecture. There remain other problems with quantification: delay of the signal, restricted diffusion through the capillary wall, and efficient labelling of arterial blood.

Despite these advantages ASL is rarely used clinically in favour of the standard gadolinium bolus tracking method. The main reason for this is the poor signal to noise ratio of the measurement - typically 20 times lower than with bolus tracking. In some cases of cerebrovascular disease where the blood is delayed ASL may not be suitable.
due to lack of signal due to the short lifetime of the signal ($T_1$). This will improve with higher field scanners which will increase the lifetime of the label. Higher fields will also increase the signal to noise of the measurement. For other applications its full potential has yet to be realised partly due to poor accuracy and precision leading to a lack of confidence in the technique. One aim of this thesis is to make improvements to the modelling of the signal and test the reproducibility of the technique, in order to go some way to changing this. The inherent problems of bolus tracking will remain whereas ASL imaging will improve with higher field strengths and improved modelling.

1.3 Why measure perfusion?

Perfusion is a fundamental biological function. The rate of blood supply to a piece of tissue is an indicator of the delivery of oxygen and nutrients. Disorders of perfusion such as heart attack and stroke constitute most of the leading causes of morbidity and mortality.

1.3.1 Cerebrovascular disease

Perfusion imaging has a broad range of potential applications in clinical neuroscience. The most obvious of these is in the characterization of cerebrovascular disorders in which there is a large change in perfusion. There is great interest in using perfusion imaging to identify brain regions with potentially reversible ischemia. Preliminary studies suggest that ASL perfusion MRI can identify regions of hypoperfusion in acute stroke [32] and chronic cerebrovascular disease [42]. ASL Perfusion MRI is particularly attractive since it can be performed serially to evaluate responses to therapy. Other techniques such as PET may be too toxic for regular measurements.

1.3.2 Degenerative disorders

Degenerative diseases of the central nervous system cause progressive reductions in regional neural function which are typically accompanied by alterations in regional perfusion. Perfusion imaging can contribute to the differential diagnosis of such disorders, or be used to assess new therapies. Perfusion changes may precede neuronal dysfunction or simply reflect it, a matter open to debate. Hypoperfusion has been reported in Alzheimer's disease [2], and in Chapter 6 of this thesis I present evidence for white
matter perfusion increase in multiple sclerosis.

1.3.3 Epilepsy

For some types of epilepsy surgical removal of the seizure focus provides dramatic relief. Regional changes in perfusion can be used for localization of seizure foci which produce ictal hyperperfusion [182] and interictal hypoperfusion [174]. For ictal imaging, SPECT provides the benefit of injecting tracer during a seizure and scanning up to several hours later [186]. Interictal metabolic imaging with FDG-PET has been most widely used for lateralization of temporal lobe epilepsy [139], though perfusion MRI is also capable of identifying mesial temporal hypoperfusion which correlated with the seizure focus [213].

1.3.4 Functional activation

Regional perfusion changes may be used as a surrogate marker for changes in regional brain function. Regions of perfusion increase during a task identify the brain regions active during that task, a very useful technique in cognitive neuroscience research. The use of perfusion imaging rather than the more commonly used BOLD imaging [148] has several advantages. BOLD contrast depends on several parameters, whereas perfusion is a single well-defined physiological parameter. Increasing evidence suggests that perfusion changes have greater spatial resolution than BOLD changes, located closer to the region of neuronal activation [47, 123, 110]. These issues are discussed further in Chapter 6.

1.4 Causes of perfusion variation in the brain

The cerebral vascular bed has great capacity for maintaining adequate blood flow in the face of varied external perturbations. This process is termed autoregulation. Blood flow is controlled through dilation and constriction of the vessel walls, in response to pressure changes, local changes in oxygen delivery or demand, and vasoactive chemicals [202]. Smaller vessels have the capacity to dilate up to double their original volume, but if further vasodilatory stimulus is applied, the vessels will dilate no further and the limit of autoregulation is reached [202]. Continuing research shows that there is no unique or predominant mechanism responsible for changes to the tension of the vessel
In general, perfusion is directly coupled to brain metabolism such that global or regional changes in neural activity are reflected in global or regional perfusion changes. Excitatory and inhibitory neurotransmission are both energy consuming processes [240], and produce increases in cerebral blood flow and metabolism. However, in certain pathological conditions there may also be relative uncoupling of blood flow and metabolism or neuronal function [240]. Although a coupling of regional perfusion and metabolism in the working brain has been recognized for over a century [175], neither the precise mechanisms nor even the purpose for this coupling are completely understood. Regional perfusion increase may be required to supply the oxygen and nutrients for increased neuronal metabolism, or to remove the potentially toxic waste products of metabolism.

1.5 Outline of thesis

Chapter 2
The basic principles of MRI are introduced. The advantages and disadvantages of bolus tracking and arterial spin labelling (ASL) are discussed.

Chapter 3
The theoretical modelling of the ASL signal is considered in more depth. The model is developed to include corrections for restricted capillary permeability to water. To achieve this the new model incorporates an extravascular and a blood compartment with the permeability surface area product (PS) of the capillary wall characterising the passage of water between the compartments. Using published values for PS the new model predicts that labelled spins spend a considerable time in the blood compartment before exchange. This makes an accurate blood $T_1$ measurement crucial for perfusion quantification; conversely, the tissue $T_1$ measurement is less important. The model gives up to 62% reduction in perfusion estimate for human imaging at 1.5T compared to the current single compartment model. Simplifications to the new two-compartment model are considered and the accuracy of these simplified models are calculated through simulations. The question of which size vessels to include in the perfusion measurement is considered.
Chapter 4
The two-compartment model is tested on Continuous ASL (CASL) data, making comparisons with other models. The new model produces a closer fit to the data over all extremes of tissue type and perfusion. The model is used to estimate capillary water permeability, arrival time and perfusion in three subjects using the CASL technique. Preliminary tests of static tissue subtraction and $T_1$ measurements are made.

Chapter 5
Measurements of perfusion in a group of normal subjects is reported. The reproducibility of the technique is established and the intra and inter-subject variability. A study on thirty-two normal subjects shows significant perfusion changes with age and gender in agreement with previous studies using PET, MRI bolus tracking and SPECT. It is found that grey matter perfusion falls by 0.5% per year, and females have a higher brain perfusion than males (about 15%). The temporal stability of perfusion is tested by scanning one subject several times over a day and a week. Long-term stability over several months is tested in five normal subjects. This information is of physiological interest and is important information if the technique is to be used clinically to measure perfusion changes during disease. Temporal and Spatial correlations in perfusion within the brain are investigated.

Chapter 6
The measurement of perfusion change is investigated. Applications of ASL in stroke, arteriovenous malformation, motor activation and a more extensive study in multiple sclerosis are reported.

Chapter 7
Conclusions and suggestions for future work.
Chapter 2

Blood perfusion measurements using MRI

The focus of this thesis is on the more recently developed techniques of perfusion imaging using MRI. In this chapter I will discuss the advantages and disadvantages of the bolus tracking and ASL techniques. Before this it will be useful to give a summary of the basic principles of MRI, including the fast acquisition technique of Echo-Planar Imaging (EPI) upon which most MRI perfusion techniques rely.

2.1 Principles of MRI

2.1.1 Classical description of nuclear magnetic resonance

2.1.1.1 Response of a proton spin in a magnetic field

Atomic nuclei have intrinsic angular momentum, \( \vec{J} \), often referred to as 'spin'. The nuclear spin can be thought of as causing an electric current and, hence, an associated magnetic moment, \( \vec{\mu} \). The relationship between these quantities [79] is given by:

\[
\vec{\mu} = \gamma \vec{J}
\]  

(2.1)

where the proportionality constant \( \gamma \) is the gyromagnetic ratio. The hydrogen proton has the largest magnetic moment of any stable nucleus and is abundant in the body, hence it is most often used in MRI. For the proton \( \gamma = 2.675 \times 10^8 \text{ rad s}^{-1}\text{T}^{-1} \).

Classically, the proton can be considered as a magnetic dipole moment with magnetic moment \( \vec{\mu} \). When a dipole is placed in an external magnetic field \( \vec{B}_0 \) it experiences a
torque \( \vec{N} \) [46] given by:

\[
\vec{N} = \vec{\mu} \times \vec{B}_0
\]

which causes the angular momentum of the dipole to change [79] according to:

\[
\frac{d\vec{J}}{dt} = \vec{N}
\]

These are fundamental equations of electromagnetism. From them I will show how it is possible to derive the frequency of precession of the dipole, the Larmor frequency.

Substituting equation 2.1 into the left hand side of equation 2.3 and equation 2.2 into the right hand side, we find:

\[
\frac{d\vec{\mu}}{dt} = \gamma \vec{\mu} \times \vec{B}_0
\]

This is the fundamental equation of motion for MRI, describing how the magnetic moment of a dipole, \( \vec{\mu} \) precesses when placed in an external magnetic field \( \vec{B}_0 \). Figure 2.1 is a geometrical representation of equation 2.4. Since the rate of change of \( \vec{\mu} \) is proportional to a cross product involving \( \vec{\mu} \) its magnitude remains constant but its direction changes. \( \vec{\mu} \) performs a left-hand rotation about \( \vec{B}_0 \). The geometry of figure 2.1 gives:

\[
d\mu = \mu \sin \theta d\phi
\]
And from equation 2.4 we have:

\[ d\mu = \gamma |\vec{\mu}| \times \vec{B}_0\,dt = \gamma \mu B_0 \sin \theta \,dt \]  \hspace{2cm} (2.6)

Comparing equation 2.5 with equation 2.6 we find \( \gamma B_0 \,dt = d\phi \), giving the formula for the frequency of precession, the Larmor frequency \( \omega_0 \):

\[ \omega_0 = \frac{d\phi}{dt} = \gamma B_0 . \]  \hspace{2cm} (2.7)

### 2.1.1.2 Resonance and the rf pulse

In an MRI experiment a second, smaller, rotating magnetic field \( \vec{B}_1 \) is applied perpendicular to the large external field \( \vec{B}_0 \). To consider the effect of this additional field it is easier to consider the system in a reference frame rotating with angular velocity \( \Omega \). In this rotating reference frame (denoted by ') the equation of motion 2.4 becomes:

\[ \left( \frac{d\vec{\mu}}{dt} \right)' = \gamma \vec{\mu} \times \vec{B}_{\text{eff}} \]  \hspace{2cm} (2.8)

where

\[ \vec{B}_{\text{eff}} = \vec{B} + \frac{\Omega}{\gamma} \]  \hspace{2cm} (2.9)

and \( \vec{B} \) is the total applied field, i.e. \( \vec{B} = \vec{B}_0 + \vec{B}_1 \). For proof see Haacke et al. [79].

Consider the application of a constant field \( \vec{B}_0 = B_0 \hat{z} \), and a left circularly polarized field \( \vec{B}_1 = B_1 (\hat{x} \cos \omega t - \hat{y} \sin \omega t) \). Consider also a rotating reference frame of angular velocity \( \Omega = -\omega \hat{z} \) about the \( \hat{z} \) axis (i.e. \( \hat{z}' = \hat{z} \)). \( \vec{B}_0 \) and \( \vec{B}_1 \) can be expressed in terms of the primed coordinates as: \( \vec{B}_0 = B_0 \hat{z}' \) and \( \vec{B}_1 = B_1 \hat{z}' \). From equation 2.9 with \( \vec{B} = \vec{B}_0 + \vec{B}_1 \) and equation 2.7 with \( \omega_i = \gamma B_i \) it follows that:

\[ \vec{B}_{\text{eff}} = [(\hat{z}' (\omega_0 - \omega) + \hat{x}' \omega_1)]/\gamma \]  \hspace{2cm} (2.10)

If the frequency of the \( B_1 \) field in the stationary reference frame, \( \omega \) is equal to the Larmor frequency \( \omega_0 \) the first term in equation 2.10 cancels giving \( \vec{B}_{\text{eff}} \) along the \( \hat{z}' \) axis only. Under these 'resonance' conditions the \( B_1 \) field is optimally chosen to tip the proton spin around the \( \hat{z}' \) axis. The angle of tip depends on the duration of the \( B_1 \) field, which is usually short, and hence called an 'rf pulse' (radio frequency pulse).

Resonance can also be understood in quantum mechanical terms. In a magnetic field a proton can occupy one of two energy states, corresponding to the nuclear spin pointing
either parallel or antiparallel to the applied field. The energy difference between these states can be expressed in terms of a frequency: \( E = \hbar \omega \), where \( \hbar \) is Planck’s constant divided by \( 2\pi \). Energy must be put in at the resonant frequency \( \omega \) in order to excite the protons from the low energy to the high energy state. The resonant frequency, measured experimentally, is found to be the Larmor precession frequency.

### 2.1.2 Relaxation and the Bloch equations

A small volume (voxel) of tissue contains many protons. A collection of proton spins all precessing at the same frequency is called an ‘isochromat’. An isochromat has an average magnetic moment density, called its ‘magnetisation’. For a volume \( V \) containing \( N \) protons with magnetic moments \( \vec{\mu}_i \), the magnetisation \( \vec{M} \) is:

\[
\vec{M} = \frac{1}{V} \sum_{i=0}^{N} \vec{\mu}_i
\]

For non-interacting protons equation 2.4 becomes:

\[
\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B}
\]

However, protons do interact, exchanging energy with the surroundings and with each other, and additional terms must be added to this equation.

#### 2.1.2.1 Spin-lattice relaxation

An isochromat will always try to occupy the lowest energy state, with the spin magnetic moments aligned with the external field. If the equilibrium magnetisation of the system is disturbed through the application of an rf pulse the magnetisation will return to its equilibrium value \( (M_0) \) if the external field is along \( \vec{z} \). The protons lose energy to the nearby lattice of atoms, with which they are in thermal contact. This relaxation is described [79] by the equation:

\[
\frac{dM_x}{dt} = \frac{1}{T_1} (M^0 - M_x)
\]

The rate of change of the longitudinal magnetisation \( M_x \) is proportional to the difference between \( M_x \) and its equilibrium value \( M^0 \). \( T_1 \) is called the ‘longitudinal relaxation time’. It is measured experimentally and can range from 700ms [75] in human white matter to 4500ms in cerebrospinal fluid [79] at 1.5T.
2.1.2.2 Spin-spin relaxation

Isochromats experience local (internal) field variations due to the small fields produced by neighbouring spins. This causes local differences in the precessional frequencies of the spins. The spins begin to lose phase coherence and the transverse magnetisation decreases. For an external field $\vec{B}$ along $\vec{z}$ a decay term due to transverse relaxation can be added to equation 2.12 to give:

$$\frac{d\vec{M}_\perp}{dt} = \gamma \vec{M}_\perp \times \vec{B} - \frac{1}{T_2} \vec{M}_\perp$$  \hspace{1cm} (2.14)

where $\vec{M}_\perp$ is the transverse component of the magnetisation. $T_2$ is called the 'transverse relaxation time' and can range from 80ms in human white matter to 2200ms in cerebrospinal fluid [79] at 1.5T. Additional dephasing can come from external field inhomogeneities and static tissue susceptibility gradients. $T_2^*$ describes the transverse relaxation due to both internal and external causes. Dephasing due to external causes can be recovered through the use of spin echoes, as described in section 2.1.5.2.

2.1.2.3 The Bloch equation

The behaviour of the magnetisation, including relaxation effects can now be fully described by a single equation:

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B} + \frac{1}{T_1}(M^0 - M_z)\vec{z} - \frac{1}{T_2} \vec{M}_\perp$$  \hspace{1cm} (2.15)

true for an external field $\vec{B}$ along $\vec{z}$ (for which $\vec{M} \times \vec{B} = \vec{M}_\perp \times \vec{B}$). This is called the Bloch equation [23] and is a combination of equations 2.13 and 2.14. $M^0$ is the equilibrium magnetisation in the $z$-direction, and $M_\perp$ is the transverse magnetisation.

For a constant external field (i.e. no rf pulse) we have $\vec{B}=B_0\vec{z}$, giving:

$$\frac{dM_x}{dt} = \omega_0 M_y - \frac{M_x}{T_2}$$  \hspace{1cm} (2.16)

$$\frac{dM_y}{dt} = -\omega_0 M_x - \frac{M_y}{T_2}$$  \hspace{1cm} (2.17)

$$\frac{dM_z}{dt} = \frac{M^0 - M_z}{T_1}$$  \hspace{1cm} (2.18)

Blood perfusion is measured by giving the blood magnetic properties and using it as a tracer. The tracers used can alter the $T_1$ or the $T_2$ of the tissue volume.

2.1.3 Signal detection

In a simple NMR experiment the sample is placed in a uniform $\vec{B}$ field along $\vec{z}$ and a 90° rf pulse is applied. After the pulse, there exist transverse ($M_x$ and $M_y$) components
of magnetisation, which begin to decay due to spin-spin relaxation. Changes in the transverse magnetisation induce an alternating voltage (at the Larmor frequency) in a receiver coil, placed to detect flux changes in the transverse plane. The transverse magnetisation, and hence the amplitude of the received signal, decays with the time constant $T_2$. This is called the free induction decay.

### 2.1.4 Image formation

#### 2.1.4.1 One-dimension

By using a magnetic field gradient in addition to the main magnetic field it is possible to create a field whose strength varies linearly with position. This was first utilized by Lauterbur [112] and is the basic concept behind imaging. Since the Larmor frequency depends on field strength, according to equation 2.7, the spins at each position will precess at a unique frequency. The free induction decay signal will now contain a spread of frequencies corresponding to the proton densities at particular positions (along one dimension) in space. We then need a means of determining the signal strength as a function of frequency; the Fourier transform. Hence the proton density from each piece of tissue can be assigned in a one-dimensional projection. In order to form an image, information in two dimensions is required, as described in section 2.1.4.3.

#### 2.1.4.2 Image slice selection

In order to collect a two-dimensional image of a slice from an object, it is necessary to collect signal from that slice only. This is achieved by the method of slice excitation proposed by Lauterbur et al. [111] and Mansfield et al. [128]. For excitation of a slice in the xy plane, for example, a field gradient is applied in the perpendicular direction (the z direction). This gives the spins in the object different Larmor frequencies depending on their location along the z axis. The rf pulse is applied with only a limited bandwidth of frequencies and so will only excite a limited range of spin frequencies, and hence spins in a specific slice of the object. The width of the slice is controlled by changing either the bandwidth of the rf pulse or the amplitude of the applied gradient.

#### 2.1.4.3 Two-dimensions

Section 2.1.4.1 described how a field gradient along one direction can be used to produce a one-dimensional projection of proton density. For a two-dimensional image a second
gradient must be applied perpendicular to the first. If a second ‘dimension’ of time existed then a two dimensional Fourier transform in the two time dimensions would produce a two-dimensional image. Of course this second time dimension does not exist. However, by repeating the application of one field gradient several times we can mimic a second time dimension. With each repetition a second field gradient is applied perpendicular to the first so that its effect increases linearly in this pseudo-time.

2.1.4.4 k space

![Diagram of spin dephasing with increasing gradient strength and duration.](image)

Figure 2.2: Spin dephasing with increasing gradient strength and duration. The two spins in red have equal k values and therefore equal dephasing. The spins in green can be detected as being in a different position to the origin since they have at least one full cycle phase shift. Increased gradient strength and duration improves the resolution.

k space [120, 201] is a useful concept in understanding the information that we collect from the free induction signal. Consider a line of protons in one dimension. As soon
as a field gradient is applied the precessional frequency of the protons change and they begin to move out of phase with each other. The larger the gradient and the longer its duration the greater the phase difference between neighbouring protons. This is best shown pictorially in figure 2.2.

\[ k = \gamma \int G(t) dt \]

As \( k \) increases, the phase shift increases. The two spins shown in red in figure 2.2 have equivalent \( k \) values and hence equivalent phases. The gradient is applied during data acquisition so that the data collected is a record of the evolving phase changes. In order to assign signal to different regions of space the Fourier transform must recognise that the protons in different regions have different frequencies. In order for a discrete Fourier transform to detect two protons as having different frequencies they must have one full cycle (360°) of phase difference [35]. This condition is only satisfied by the spins in green in figure 2.2 and will therefore be the only spins that can be detected as being at a different position from the spin at the origin. Larger \( k \) values represent greater phase differences and therefore the distance between protons with a full cycle phase difference is smaller - the resolution is increased. In order to achieve an image of the required resolution a minimum distance in \( k \) space must be covered. Low values in \( k \) space correspond to low spatial frequencies and higher values to high spatial frequencies in the image.

### 2.1.5 Echo Planar Imaging

The imaging sequence is a repetitive protocol that allows complete sampling of \( k \) space (to the desired resolution) in two dimensions. Recently, due to the use of high gradient amplitudes, fast gradient switching and fast sampling this whole process can be completed before the MR signal has decayed away, typically less than 40 ms. This method of collecting a complete image from one excitation is called Echo-Planar Imaging or EPI [127].

Figure 2.3 shows the imaging sequence for EPI and figure 2.4 the \( k \)-space trajectory that it encodes. In figure 2.3 the rf pulse, which rotates the magnetisation by an angle \( \alpha^\circ \), is applied in the presence of the slice-select gradient G-Select. This excites spins in a specific slice of the object and produces the free induction decay, point A in figure 2.4 at the centre of \( k \)-space. Negative readout and phase gradients are applied and the \( k \)-space trajectory moves to point B in figure 2.4. There then follows a sequence of brief
Figure 2.3: EPI pulse sequence. Protons in a single slice are excited by transmitting the RF pulse in the presence of G-select, a gradient on the slice selective axis. From [35].

Figure 2.4: $k$ space trajectory encoded by the pulse sequence of figure 2.3. The direction of $k_{\text{readout}}$ traversal is reversed by switching the polarity of the readout gradients $G_{\text{readout}}$. The pulses of $G_{\text{phase}}$ move the trajectory up line by line through $k_{\text{phase}}$. From [35].

Phase-encoding gradient pulses followed by a longer readout gradient until the whole of $k$-space in both dimensions $k_{\text{readout}}$ and $k_{\text{phase}}$ is sampled. The image created will show the magnitude of the longitudinal magnetisation of the tissue at the time just before
the $\alpha^\circ$ pulse. The faster the image is collected the more accurate this will be due to reduced transverse dephasing and therefore reduced $T_2^*$ weighting.

### 2.1.5.1 Gradient echo EPI

The pulse sequence above describes gradient echo EPI. This refers to the use of pairs of negative then positive gradients which dephase and then rephase the transverse magnetisation. However, the gradient reversal refocusses only those spins that have been dephased by the action of the gradient itself. Specifically, phase shifts resulting from magnetic field homogeneities or static tissue susceptibility gradients are not cancelled at the centre of the gradient echo. Thus, the gradient echo signal intensity is dependent on $T_2^*$ relaxation.

### 2.1.5.2 Spin echo EPI

Spin echo EPI refers to the gradient echo EPI readout as described above but with a spin echo preparation. In a spin echo sequence [80] a 90° rf pulse is applied to tip the magnetisation into the transverse plane, along, say the $y'$ axis. The spins begin to dephase due to spin-spin relaxation. A second rf pulse is applied with a tip angle of 180° which rotates the magnetisation onto the -$y'$ axis. The spins begin to rephase and the transverse magnetisation is re-established. An 'echo' is formed. the time between the initial rf pulse and the echo is called the echo time (TE). The phase shifts resulting from magnetic field inhomogeneities and static tissue susceptibility gradients are cancelled and the signal is dependent on $T_2$ relaxation. With spin echo EPI the spin echo is tagged onto the front of the EPI readout, and is carefully timed such that the echo occurs at $k=0$. Since the $k=0$ line determines the bulk of the image contrast, the EPI image is largely $T_2$ weighted.

### 2.2 Bolus tracking

Bolus tracking involves the use of an exogenous tracer injected intravenously. The most common tracer is the paramagnetic contrast agent gadolinium diethylene triamine pentaaacetic acid which I will refer to as 'gadolinium'. A bolus of the tracer is injected into a vein and some time later (10-20 secs) the bolus passes through the vasculature of the brain. Gadolinium changes the $T_1$, $T_2$ and $T_2^*$ relaxation times of the tissue through which it passes. The change to $T_1$ is small since only a small proportion of
the protons come into contact with the intravascular tracer. The $T_2$ and $T_2^*$ effects are larger. Gadolinium has many free electrons and when placed in an external magnetic field it will produce its own local field. In a voxel, although the tracer is confined to many small vessels, the local fields will extend outside these vessels [63, 205] and produce heterogeneity in the magnetic field across the voxel. These static inhomogeneities cause increased dephasing of the transverse magnetisation, reducing $T_2$. This causes signal loss in gradient echo images. In spin echo images the static inhomogeneities are refocussed. However, if the echo time is sufficiently long, there are diffusion mediated susceptibility losses [96]. Water molecules will diffuse through different magnetic fields causing the water protons to precess at a range of frequencies producing dephasing and reducing $T_2$. The gradient echo losses are larger than spin echo losses and so a gradient echo EPI sequence is usually used.

![Diagram of signal drop due to $T_2^*$ change](image)

Figure 2.5: Bolus passage. Schematic diagram of signal drop due to $T_2^*$ change during the bolus passage of an intravascular paramagnetic tracer through the vasculature.

As the bolus travels through the vasculature the signal is seen to drop as shown schematically in figure 2.5. The signal drop depends on the change in $T_2^*$ (either $T_2$ or $T_2^*$) according to:

$$S(t) = S(0)e^{-TE\Delta R_2^*(t)}$$  \hspace{1cm} (2.19)

Where $R_2^*$ is the reciprocal of $T_2^*$ and TE is the echo time of the imaging technique. $\Delta R_2^*$ is the change in $R_2^*$ from baseline as the tracer passes through the tissue. $S(t)$ is the signal at time $t$ after bolus injection and $S(0)$ is the baseline signal before the tracer arrival. It has been shown both empirically [205, 172, 83] and using simulations [208, 56, 25, 95] that the reduction in $R_2^*$ is approximately linear with tracer concentration,
where $k$ is an unknown constant which depends on field strength, the type of imaging technique and the vessel architecture. Therefore a measure of $R_2^*$ reduction as the bolus passes through the microvasculature provides us with a measure of the local tissue tracer concentration curve $C(t)$. For accurate characterisation of the signal curve, images must be collected with high temporal resolution. EPI allows temporal resolutions of less than 1 second.

### 2.2.1 Non-diffusible tracer kinetics

The model used for perfusion quantification uses basic principles of tracer kinetics [239, 238, 6] for non-diffusible tracers. The central assumption is that the tracer remains intravascular. The tissue tracer concentration $C(t)$ can be expressed as a convolution [31, 152]:

$$C(t) = \int \frac{f}{\kappa_H}(C_a(t) \otimes R(t))$$  \hspace{1cm} (2.21)

where $f$ is perfusion, $C_a(t)$ is the concentration of the tracer entering the voxel, $R(t)$ is the residue function, and $\kappa_H$ is a correction factor for the difference in haematocrit between capillaries and large vessels. The residue function $R(t)$ is the fraction of tracer remaining in the voxel at time $t$ following the injection of an ideal bolus at time $t=0$. Therefore, at $t=0$, $R(t)=1$. The form of $R(t)$ in vivo remains unknown [163]. The expression for $C(t)$ given by Calamante et al. [31] includes an extra term $\rho$, the brain density (in g/ml). However, if perfusion is expressed in terms of ml min$^{-1}$ (100 ml tissue)$^{-1}$ rather than ml min$^{-1}$ (100 g tissue)$^{-1}$, this is not required.

$C(t)$ can be measured in terms of an $R_2^*$ change as described above. By locating an image voxel over a feeding artery it is also possible to measure the arterial tracer concentration curve $C_a(t)$. It is usually assumed that the arterial concentration-time curve is the same for all tissue volumes and can be approximated by a measurement in a distant large vessel, as described in the next section. By deconvolving $C_a(t)$ from $C(t)$ it is possible to find the function $fR(t)$. Various methods of deconvolution have been investigated with the method of single value decomposition (SVD) proving the most favourable, reproducing the most accurate perfusion values independently of vascular
structure and blood volume [152, 153]. After deconvolution, perfusion is the value of the function \( f_R(t) \) at \( t=0 \), when \( R(t)=1 \).

### 2.2.2 Errors in quantification

**Delay and dispersion**

One problem in performing the deconvolution is the delay time between the arterial and tissue signal. This must be corrected for if the function \( f_R(t) \) is to be found accurately. If this is not done the peak of the arterial curve will be shifted with respect to the tissue peak producing errors to the function \( f_R(t) \). Perfusion must be found from the maximum of the deconvolved curve, not the value at \( t=0 \). Another problem is the dispersion of the bolus after measurement of \( C_a(t) \) before it reaches the volume of interest. If uncorrected this will lead to underestimation of perfusion. This error can be minimised by measuring \( C_a(t) \) in a voxel as close as possible to the volume of interest.

**Measurement of tracer concentration**

The exact relationship between the concentration of tracer and \( R_2^* \) change is not known, i.e. the value of \( k \) in equation 2.20 is unknown. Some have assumed that the relationship is the same for \( C(t) \) and \( C_a(t) \) [168, 178], cancelling the relation constant \( k \) during deconvolution. This assumption is wrong since the relationship is dependent on the size and architecture of the vasculature, which is clearly different for \( C(t) \) (capillaries) and \( C_a(t) \) (artery). However Rempp et al. have shown that the error is small [168]. Without this assumption, an alternative method of quantifying perfusion is to use an internal calibration, for example using prior knowledge of perfusion in one tissue (usually white matter) and scaling other measurements accordingly. This perfusion value is known from published values for the chosen tissue [153] or can be measured using another technique [150, 151, 217].

**Tracer leakage**

Applications are limited due to the assumption that the tracer is intravascular which is not the case outside the brain or in case of blood-brain barrier breakdown. The main error is not however due to the consequent inaccuracies of the theory but due to leakage causing an increase in the \( T_1 \) relaxation rate, opposing the \( T_2 \) signal loss. This will depend on the \( T_1 \) weighting of the pulse sequence. There are several solutions to this problem. First, the effect can be reduced by reducing the \( T_1 \) weighting of the sequence.
Alternatively, the use of a dysprosium-based tracer [107] reduces the error due to its much smaller $T_1$ enhancement than gadolinium. The leakage can be reduced through the use of macromolecular contrast agents, such as albumin bonded to gadolinium, which remain intravascular without the blood-brain barrier [190]. These new tracers may soon be available for human use. Another solution is to model the effect of the leakage [196].

Large vessel contribution
The measurement of true perfusion is in question due to the inclusion of signal from large vessels. Flow of tracer through all vessels will cause a change in signal and will contribute to the tissue concentration curve $C(t)$. As a result the measurement is a mixture of perfusion and larger vessel flow, resulting in an overestimation of perfusion. One method of homing in on microvasculature flow is by using spin echo imaging instead of gradient echo. Simulations show that the susceptibility contrast in gradient echo images arises from both large and small vessels [25, 95, 208] and is fairly independent of vessel size. With spin echo the signal change reaches a maximum for capillaries and decreases with increasing vessel size [25, 208]. This is because the signal loss with spin echo is a result of water diffusion through regions of different susceptibility. With smaller vessels more water molecules will experience changing susceptibility and the signal loss will increase. However, some contamination from larger vessels will remain.

Measuring the arterial input function
Aside from these theoretical issues there are also practical problems in the implementation of the technique. One difficulty is the simultaneous measurement of the arterial and tissue signal-time curves. There are two approaches; the first, devised by Perman et al [162], uses a dual echo FLASH sequence which collects two images at different echo times within the same repetition time. One of these can be positioned through the carotid artery and the other in the slice of interest. A region on (or around) the carotid artery can be identified by eye or by computer [168] and the signal-time curve recorded. The second approach is to image only the slice of interest and pick out voxels in major vessels from which to extract the arterial signal-time curve [153]. The problem here is that vessels may not be obvious and voxels may be hard to find. Partial volume effects with surrounding tissue may cause $C_a(t)$ to be underestimated, thereby overestimating perfusion.
Bolus injection

Other practical difficulties arise in the administration of the bolus. The bolus must be injected very rapidly (lasting no more than 10 secs) into a vein (usually in the arm) such that it remains as a bolus after transit through the heart and the microvasculature of the lungs. It must also be injected at a steady rate to avoid the problem of secondary peaks in the bolus. The use of a power injector can alleviate some of these problems.

In summary, this method provides us with large signal changes (about 20%) that can be used to estimate perfusion with the use of an internal calibration. The method is easily amenable to multislice imaging. As well as perfusion it is possible to estimate two other useful parameters, blood volume and the mean transit time of blood through the vasculature. In many applications the pursuit of true perfusion quantification is abandoned in favour of the use of summary parameters such as time to peak and peak height of the bolus passage. These are easy to measure and are clinically useful, but have been recently shown to be highly dependent on the arterial tracer concentration and the tissue residue function [163]. On the down side, the technique requires fast intravenous injection of a contrast agent which necessitates a period of waiting for washout before repeat imaging. The side effects of the tracers may also limit the frequency of repeat measurements. Another disadvantage with this method is the lack of a true capillary perfusion measurement due to signal contamination from larger vessels. Quantification is poor which hinders accurate comparisons of perfusion between images taken at different time points and between different subjects.

2.3 Arterial spin labelling

Arterial spin labelling techniques can be split into two categories - continuous ASL or CASL and pulsed ASL or PASL. Within pulsed ASL there are a number of further variations. For all techniques the approach is to create an endogenous tracer by changing the magnetisation of the blood, or 'labelling' the blood. Two images are collected using EPI, one with the blood labelled and one without, such that on subtraction the static tissue signal is removed and only signal from the labelled blood remains.
Figure 2.6: Continuous Arterial Spin Labelling. Blood water protons are inverted or ‘labelled’ as they flow through the labelling plane. Control labelling uses double inversion to leave the blood unlabelled. On subtraction (control - labelled) the signal comes only from labelled water protons.

2.3.1 Continuous arterial spin labelling

Continuous ASL uses an applied rf field to continuously saturate [43] or invert [216] the longitudinal magnetisation of the arterial blood protons entering the brain (see figure 2.6). Continuous inversion provides double the magnitude of label than saturation and was used in all later developments of the technique. Inversion is achieved by a process called adiabatic fast passage [45] which makes use of the linear motion of the blood water protons in the arteries. A field gradient $G$ is applied along the direction of the constant magnetic field, with which the major feeding arteries are roughly aligned. Protons in the arteries flow with velocity $v$ through this field gradient and their resonant frequency increases. A continuous low-power rf field $B_1$ is applied for a few seconds, perpendicular to the field gradient, such that the rf field is on resonance in a plane through the neck, called the inversion plane. As the flowing protons pass through resonance their magnetisation is inverted. Inversion is only achieved if:

\[
\frac{1}{T_1}, \frac{1}{T_2} \ll \frac{Gv}{B_1} \ll \gamma B_1 \tag{2.22}
\]

which must be satisfied for a range of velocities. Failure to satisfy the left inequality results in $T_2$ relaxation during the inversion of the magnetisation. If the right inequality is not satisfied the inversion is less than 180° [125].
On arrival at the image slice the magnitude of the longitudinal magnetisation of the arterial blood has decreased (i.e. Mz has become less negative) due to T1 relaxation but is nevertheless different from the fully relaxed magnetisation of the tissue. The arterial blood thus reduces the longitudinal magnetisation in the slice. At the end of labelling a short delay is introduced and the longitudinal magnetisation in the slice is measured using a snapshot imaging technique such as EPI. A control image is taken with a double inversion pulse to leave the blood protons effectively un-inverted. The double inversion is required (rather than nothing) to correct for magnetisation transfer (MT) effects as described in section 2.3.4.1.

Figure 2.7: The dynamics of the CASL difference signal (i.e. control - labelled) showing the passage of the labelled spins through the microvasculature. The curve shows three phases: during part 1 the signal is zero since the labelled protons have yet to reach the tissue voxel; part 2 the signal increases as labelled protons enter the voxel; part 3 the signal decreases after the end of labelling as labelled protons leave the tissue voxel and decay due to longitudinal T1 relaxation.

Figure 2.7 shows the tissue magnetisation of the difference image (control - labelled) with time after the start of labelling. There are 3 parts to this curve: part 1 has no signal since the labelled blood has yet to reach the tissue volume; part 2 shows rising signal as the labelled blood continues to enter the slice; and part 3 shows decaying signal due to longitudinal relaxation and outflow after the end of incoming arterial signal.

When applying this technique on a clinical scanner hardware problems limit the
application of a continuous rf pulse. Specifically, the limits on the duty cycle of the rf amplifiers and the specific absorption rate of the sequence can be exceeded. The duty cycle is reduced by applying the labelling pseudo-continuously in short blocks [170] of roughly 200 ms with a short 5ms gap [3].

2.3.2 Pulsed arterial spin labelling

In contrast to continuous inversion through a plane, pulsed ASL techniques label a large volume of spins using a short rf pulse. Figure 2.8 shows the three principal pulsed techniques with the shaded areas representing the coverage of the inversion pulse. EPISTAR (Echo Planar Imaging and Signal Targeting with Alternating Radio frequency) was the first pulsed technique proposed by Edelman in 1994 [50]. For the labelled image a single 180° rf pulse is applied in the presence of a field gradient to invert the longitudinal magnetisation of the protons in a slab inferior to the image slice. After an inversion time an echo-planar image is taken. The signal will include a contribution from labelled blood water flowing into the image slice. In the control image an inversion slab is placed superior to the image slice, in order to cancel magnetisation transfer effects. A variant named PICORE [222] (Proximal Inversion with Control for Off-Resonance Effects) simply switches off the slab selective gradient for the control image. Note that there is a gap between the image slice and the inversion slab, which is required to avoid imperfect slice profile effects. Any contribution coming from blood in this gap and below the inversion slab will be lost.

Variations on EPISTAR include the popular technique of FAIR (Flow-sensitive Alternating Inversion Recovery) proposed around the same time by Kim, Kwong and Schwarzbauer in 1995 [102, 108, 180]. This involves differences in the geometry of the inversion slab and will include contributions from blood flowing into the slice from above.

Another popular technique is QUIPSS II (Quantitative Imaging of Perfusion using a Single Subtraction) proposed by Wong in 98 [220]. This improvement can be used with any labelling strategy and involves the saturation of the inverted region a short time after inversion. This acts to chop off the tail of the perfusing bolus, reducing the arrival time sensitivity of the technique. Arrival time is the time taken for labelled blood to travel from the labelling plane to the image slice. If the image is collected
Figure 2.8: Pulsed arterial spin labelling. Short labelling pulses invert or ‘label’ large volumes of blood and tissue protons, shown by the shaded regions. Different labelling strategies produce difference images (labelled - control) with signal only from labelled blood protons that flow into the image slice. FAIR includes contributions from blood flowing into the slice from above. QUIPSS II saturates the inverted region a short time after inversion, which chops off the tail of the perfusing bolus, reducing the arrival time sensitivity of the technique.
after the whole bolus has entered the image slice then it does not matter how long the bolus took to reach the slice.

\[
\begin{array}{c}
\text{FAIR} \\
% \text{signal change} \\
\end{array}
\]

\[
\begin{array}{c}
\text{time after start of labelling (secs)} \\
0 \quad 1 \quad 2 \quad 3 \quad 4 \\
0 \quad 0.2 \quad 0.4 \quad 0.6 \quad 0.8 \quad 1 \\
\end{array}
\]

Figure 2.9: The dynamics of the FAIR difference signal (i.e. labelled - control) showing the passage of the labelled spins through the microvasculature.

Figure 2.9 shows the tissue magnetisation of the FAIR difference image (labelled - control) with time after labelling (the inversion time). The signal in the difference image for pulsed ASL techniques is theoretically about half that of the CASL technique. This signal advantage is reduced due to longer arrival times for the continuous technique causing loss of labelling before the blood water protons reach the image slice.

2.3.3 Quantification

Accurate perfusion measurements require careful consideration of both data collection and analysis. During data collection care must be taken to include signal from all of the perfusing blood and remove all non-perfusion contributions. Data analysis must use an accurate model to correct for signal change from non-perfusion factors, which cannot be removed during data collection. These measures must be robust enough to cope with more extreme changes during disease.

A typical CASL protocol is shown in Fig. 2.10. The labelling is applied for 2 s, there is a post-labelling delay of 1 s followed by single-shot gradient echo EPI all within a repetition time of 4 s. This is then repeated with control labelling. The pair-wise imaging is then repeated around 50 times in order to improve the signal to noise ratio of
the measurement. The EPI collection must use minimum echo time in order to reduce the T2* sensitivity of the scan. The labelling plane should be positioned perpendicular to the direction of blood flow in the major feeding vessels. These include the carotid and vertebral arteries as shown on figure 1.2 of Chapter 1. Assuming signal from static tissue is removed, the subtraction image (control - label) contains signal only from perfusing blood.

To convert the difference image into a perfusion map an accurate model of the system is required. The difference signal is dependent on a number of factors: inversion efficiency, blood $T_1$, tissue $T_1$, capillary permeability, arrival time, and perfusion. Figure 2.11 shows how some of these factors can affect the signal curve. These factors can either be assumed or measured with most applications measuring only tissue $T_1$ in a separate inversion recovery experiment. There are many models to choose from with different degrees of complexity and which to use is still in debate. Early models assumed the free diffusion of water in a well-mixed tissue compartment [43, 108]. Recently this has been shown to be a poor assumption [156, 189, 237] calling for the use of a two-compartment model with separate blood and tissue compartments or the inclusion of an extra tissue transit time [4]. The downside is that these more complex models introduce extra terms into the model that must either be assumed or measured. However, neglect of arrival time or capillary permeability for example can lead to large errors in perfusion of the order of 50%. One of the most important factors in perfusion quantification is the value for the $T_1$ of blood. An accurate measurement of blood $T_1$ is difficult due to the movement of the blood. Recent measurements [187] give human blood $T_1$ of 1.4 s at 1.5 T, increasing to 1.7 s in the capillaries due to reduced haematocrit. These issues are discussed and developed in Chapter 3.

Figure 2.10: CASL protocol
Figure 2.11: The effect of perfusion, arrival time and capillary permeability on the difference signal using CASL (using equations 3.22, 3.23 and 3.24 from Chapter 3). The original signal curve represents a perfusion rate of 60 ml blood min\(^{-1}\) (100 ml tissue\(^{-1}\)), arrival time of 0.5 s and capillary wall permeability surface-area product (PS) of 1.5 ml water min\(^{-1}\) (ml tissue\(^{-1}\)). PS was increased to 10 ml water min\(^{-1}\) (ml tissue\(^{-1}\)), perfusion reduced to 30 ml blood min\(^{-1}\) (100 ml tissue\(^{-1}\)) and arrival time increased to 1 s respectively for the other curves. One parameter was altered at a time, leaving the others at their original values.

### 2.3.4 Errors in quantification

Errors in perfusion quantification can be summarised in two groups: signal change that is not from perfusing blood (non-perfusion contributions) and the loss of signal from true perfusing blood.

#### 2.3.4.1 Non-perfusion contributions

**Arterial signal**

In a typical tissue voxel there will be a range of many vessel sizes, not all of which contain blood that will perfuse this tissue. The blood in the larger vessels is destined to perfuse tissue further downstream and so the signal from this blood must be removed. There are currently two approaches to removing signal from unwanted vessels. The
first idea, developed by Ye [230] is to add a gradient pair that aim to ‘crush’ the signal from moving arterial water spins. For a spin echo sequence a pair of gradients of the same polarity are placed on either side of the 180° refocussing pulse. For a gradient echo sequence a pair of gradients of opposite polarity are placed after the 90° rf pulse before signal acquisition. During the application of the first gradient there is a phase shift of the transverse magnetisation of the spins, dependent upon their position. The second gradient reverses the phase shift for stationary spins but produces net dephasing for moving spins. A ‘b’ value [113] characterises the sensitivity of the sequence to spin movement and so determines the extent of the ‘crushing’. In theory if we could crush all of the signal from blood flowing above a certain velocity we could sensitise the measurement to capillary perfusion only. This is not possible since the gradients will cause partial signal loss from all vessel sizes [58] making this a fairly crude tool for quantification. The second method is to introduce a delay time between the end of labelling and imaging [4]. This gives time for blood in larger vessels to wash straight through the voxel and so be excluded from the measurement.

**Venous signal**

For the pulsed techniques venous blood is labelled as well as arterial blood since both arteries and veins are present in the labelling volume. If the blood in a vein then flows into the image slice it will erroneously contribute to the perfusion signal. CASL avoids this problem since only high velocity arterial blood is labelled. The position of the labelling plane near the neck, distal from the image slices means that if venous blood is labelled it will only flow away from the image slices.

**Static tissue signal - MT Effects**

The long labelling pulse with CASL can cause substantial magnetisation transfer effects in the static tissue of the image slice. Although no direct saturation of the water magnetisation in the imaging slice occurs due to the narrow linewidth of the free water peak, saturation of the macromolecular spins does occur. This results in attenuation of the free water signal via magnetisation transfer (MT) [214]. It is important that the control image has the same MT effect so that it will cancel on subtraction. One solution is to use a separate coil for labelling [235] which removes the MT effects, but this requires special hardware. For single slice applications the control plane can be placed above the head, equidistant from the image slice as the labelling plane [43]. This will
produce identical MT effects as for the labelled image, and so they will be removed on subtraction. This is not an option for multislice imaging since the MT effects are only cancelled at one slice location. Instead the control image can be taken with a double inversion pulse leaving the blood water effectively un-inverted. This is achieved through the use of amplitude-modulated control inversion [3] which mimics the MT effects of the normal labelling. For PASL, removal of the field gradient during the control image or placing the control labelling superior to the image slice mimics the MT effects.

2.3.4.2 Loss of perfusion signal

Efficient labelling

In order to include signal from all perfusing blood it must all be labelled. For the CASL technique the degree of labelling in the major supplying arteries depends on blood velocity and the angle of the vessel to the labelling plane. This is discussed further in Chapter 4 section 4.3. The PASL techniques label a large volume of blood vessels with differences in the proportion of spins labelled in different subjects likely to be small. However some measure of labelling efficiency is still required. With PASL there is an additional problem due to the limited extent of the labelling slab. The labelling slab can only extend as far as the head coil and a gap must be present between the image slice and the labelling slab to avoid errors due to imperfect slice profiles of the labelling pulses. Blood outside the labelling slab will not be labelled and will not contribute to the perfusion measurement. This problem is exacerbated with multislice imaging since the volume of unlabelled blood is increased.

Arrival time

The label will begin to decay during the time it takes the labelled blood to reach the image slice, the arrival time, which will be longer for CASL than PASL. The arrival time will be different for different brain regions and could be greatly increased in disease such as stroke. It is difficult to accurately measure the arrival time and requires several measurements at different delay times, which will greatly increase the imaging time. Another approach is to reduce the sensitivity of the measurement to arrival time. This is the aim of the pulsed technique QUIPSS II [220]. For CASL, arrival time sensitivity can be reduced by introducing a delay time after labelling before signal acquisition [4]. These techniques are especially important for multislice acquisition with large arrival time differences between slices. In stroke the arrival time might be so long that practi-
cally all the signal has decayed, a problem that can only be improved with higher field strengths to extend the lifetime of the label.

2.3.5 Future improvements in ASL

A major challenge for ASL perfusion MRI in cerebrovascular disease is to accurately measure low levels of perfusion occurring in conjunction with extremely delayed arterial arrival times. The introduction of higher field scanners will be a great benefit due to the lengthening of T₁ causing the signal to decay more slowly. For example, after an arrival time of 2 seconds at 1.5T (with a blood T₁ of about 1.4s) there is little signal left (only 24% compared to zero arrival time). At 4T the blood T₁ is increased to about 1.8s producing a signal of 33% (an increase of 37% compared to 1.5T). For other applications the increase in signal due to longer T₁ will allow higher resolution images. Resolutions of 1 mm³ have been achieved at 3 Tesla [13]. However, at higher fields, labelling efficiency may be reduced due to decreased rf amplitude in order to comply with power deposition constraints. Progress is being made in producing more efficient labelling for use at higher fields [1].

There are a number of recent improvements to labelling strategies, which aim to solve some of the remaining technical problems. These include efforts to further remove magnetisation transfer effects [93, 215] and provide more accurate multislice PASL imaging through the use of sharper slice profiles [59, 231]. Alternative image collection techniques to EPI could reduce magnetic susceptibility artefacts [33] and reduce T₂* weighting through the use of shorter echo times, for example with spiral imaging. Methods of background suppression remove the need for a control image [49] by using a series of inversion pulses to suppress the static tissue signal, thus improving temporal resolution. Improved temporal resolution will extend the usefulness of perfusion-based fMRI studies [219]. Another major advance is the use of selective labelling of individual vessels [233]. Zaharchuk et al. have achieved labelling of individual carotid arteries through the use of a small surface coil for labelling. This could be useful clinically in order to see the perfused territory of a single vessel. This approach should be useful in determining structure-function relationships in carotid stenosis and for evaluating redistribution of blood flow through the circle of Willis.

The field would greatly benefit from a standardised model so that comparisons can
be made between measurements on different sites. Testing of various models are investigated in Chapters 3 and 4. Validation of the technique by comparisons to other techniques [237, 207] and through reproducibility tests [57, 157] are important to increase confidence in the technique. Reproducibility and normal perfusion variation are the subjects of Chapter 5.
Chapter 3

Modelling the ASL signal

Many applications of ASL use the difference signal as a measure of perfusion. This is inaccurate since, as described in the previous chapter (figure 2.11), many other factors also contribute to the signal. To measure perfusion accurately we must include all contributions in a realistic model. In this chapter I consider current models of the perfusing system and extend these models to correct for restricted vessel permeability and large vessel contamination.

3.1 Mathematical approaches

There have been two main approaches to the modelling of ASL data. The first, originally proposed by Detre [43], extends the original Bloch equations to include perfusion terms. The second approach, introduced by Buxton [29], considers the signal in the difference image to come from a bolus of labelled spins travelling through the vasculature, mathematically convolved with a tissue residue function – similar to the gadolinium bolus tracking model described in section 2.2.1.

3.1.1 Bloch approach

The imaging voxel is assumed to be a single, well-mixed compartment with intra and extra-vascular water in perfect communication. Labelled water enters and leaves with perfusion rate f and relaxes with tissue longitudinal relaxation time $T_1$ as shown in figure 3.1.

The Bloch equation is modified to include the effects of incoming arterial magnetisation ($m_a$) and outgoing venous magnetisation ($m_v$). The rate of change of longitudinal
Figure 3.1: Schematic diagram of the single compartment model. Parameter definitions are given in Table 3.1 at the end of the chapter.

magnetisation, $M$, with time $t$ in the tissue voxel can be described as:

$$\frac{dM(t)}{dt} = \frac{M^0 - M(t)}{T_1} + f m_a(t) - f m_v(t) \tag{3.1}$$

This equation is generally true for all types of labelling. $M^0$ is the longitudinal equilibrium magnetisation of the tissue. For a full definition of the parameters see Table 3.1 at the end of the chapter. It is assumed that the labelled blood water molecules immediately equilibrate with the extravascular water molecules such that water in the blood leaving the voxel contains labelled molecules at the same concentration as water in the tissue voxel, weighted by the increased water concentration of blood to tissue. i.e. $m_w(t) = M(t)/\lambda$ where $\lambda$ is the brain:blood partition coefficient for water (i.e. ratio of water contents). Considering the magnetisation of the difference image we have from Eq. 3.1:

$$\Delta \frac{dM(t)}{dt} = \Delta \left( \frac{M^0 - M(t)}{T_1} \right) + \Delta (f m_a(t)) - \Delta \left( \frac{f M(t)}{\lambda} \right) \tag{3.2}$$

Where $\Delta$ represents the change in signal between the two images, i.e. with labelling minus control. Assuming that the physical constants $f$, $T_1$, $M^0$, and $\lambda$ do not change between the two scans we have:

$$\frac{d\Delta M(t)}{dt} + \frac{\Delta M(t)}{T_{1_{app}}} = f \Delta m_a(t) \tag{3.3}$$

where

$$\frac{1}{T_{1_{app}}} = \frac{1}{T_1} + \frac{f}{\lambda} \tag{3.4}$$
Magnetisation is a measure of the magnetic dipole moment density and so the difference in magnetisation between the two images $ΔM(t)$ can be thought of as a measure of the concentration of labelled spins in the difference image. Equation 3.3 is a straightforward first order differential equation that can be solved provided we know $Δm_a(t)$, the difference in arterial magnetisation between the label and control image. The form of $Δm_a(t)$ will depend on the type of labelling used. For a FAIR [102] experiment with labelling at $t=0$ we have $Δm_a=2m_0α\exp(-t/T_{1b})$ for $t≥0$ and $Δm_a=0$ for $t<0$, where $m_0^o$ is the equilibrium magnetisation of arterial blood, $α$ is the inversion efficiency of the labelling and $T_{1b}$ is the $T_1$ of blood. The factor 2 comes in because we are considering the difference in magnetisation between the control and labelled inverted images which is twice the equilibrium magnetisation at $t=0$. Using this expression for $Δm_a$ in Eq. 3.3 and solving for $ΔM(t)$ we get the familiar result for FAIR [108]:

$$ΔM(t) = 2f_0α \left[ e^{-\frac{t}{T_{1app}}} - e^{-\frac{t}{T_{1a}}} \right]$$ (3.5)

This is the same solution as derived when considering the two sequences separately and subtracting [108]. For a CASL [3] experiment with the start of labelling at $t=0$ we have $Δm_a(t)=2m_0α\exp(-t/t_1b)$ for $t_A≤t≤t_A+t_L$ and $Δm_a=0$ for $t < t_A$ and $t > t_A+t_L$. Using this expression for $Δm_a$ in Eq. 3.3 and solving for $ΔM(t)$ we get:

$$ΔM(t) = 0 \quad (t < t_A)$$ (3.6)

$$ΔM(t) = 2f_0αT_{1app}e^{-\frac{t_A}{T_{1app}}}e^{-\frac{t}{T_{1app}}} \left( 1 - e^{-\frac{t_A}{T_{1app}}} \right) \quad (t_A≤t≤t_A+t_L)$$ (3.7)

$$ΔM(t) = 2f_0αT_{1app}e^{-\frac{t}{T_{1app}}}e^{-\frac{(t-t_A)}{T_{1app}}} \left( e^{\frac{t_A}{T_{1app}}} - 1 \right) \quad (t > t_A+t_L)$$ (3.8)

Figure 3.2 shows these solutions plotted as $ΔM(t)/m_0^o$ as a function of $t$, the time after the start of labelling using typical parameters for human grey matter at 1.5T from Table 3.2 at the end of the chapter. It can be seen that CASL has the theoretical advantage with a larger signal difference. In practice this will be reduced due to longer arrival times than the 0.5 s used in this simulation.

### 3.1.2 Bolus approach

An alternative approach is to consider the difference image directly assuming all static tissue signal is subtracted out. The signal comes from the bolus of labelled blood passing through the vasculature. The magnetisation in the tissue is found by convolving the arterial magnetisation $Δm_a(t)$ with a magnetisation relaxation function $m(T)$ and a
Figure 3.2: Typical signal curves from two ASL techniques, FAIR and CASL. The y axis shows signal change which is the difference between the labelled and control images normalised to the equilibrium arterial blood magnetisation, $m_0^A$. The x axis is the time after the start of labelling which for FAIR is equivalent to the inversion time and for CASL is equal to the labelling time plus delay time.

residue function $r(T)$, as described by Buxton et al. [29]. $m(T)$ describes the fraction of longitudinal magnetisation remaining after decaying for time $T$ and $r(T)$ describes the fraction of molecules remaining in the voxel at time $T$ after arrival. The convolution can be written mathematically as:

$$\Delta M(t) = f \int_0^t \Delta m_a(\tau) r(t-\tau) m(t-\tau) d\tau$$  \hspace{1cm} (3.9)

If we assume that the magnetic decay and the washout of labelled water are exponential in form, then $m(T)=e^{at}$ and $r(T)=e^{bt}$, where $a$ and $b$ are unknown constants. Using the exponential product rule $m(t-\tau)=m(t)m(-\tau)$ and $r(t-\tau)=r(t)r(-\tau)$. This gives:

$$\Delta M(t) = f r(t)m(t) \int_0^t \Delta m_a(\tau) r(-\tau) m(-\tau) d\tau.$$  \hspace{1cm} (3.10)

Differentiating the right hand side as a product this gives:

$$\frac{d(\Delta M(t))}{dt} = f \frac{d(r(t)m(t))}{dt} \int_0^t \Delta m_a(\tau) r(-\tau) m(-\tau) d\tau + f r(t)m(t) \Delta m_a(t) r(-t) m(-t).$$  \hspace{1cm} (3.11)

Since $m(T)$ and $r(T)$ are exponential it follows that $m(t)m(-t)=1$, and the same for $r(t)$. Using this, the right hand term reduces to $f\Delta m_a(t)$. The left hand term is also simplified by using $d(r(t)m(t))/dt = (a+b)r(t)m(t)$. This gives:

$$\frac{d(\Delta M(t))}{dt} = (a+b)\Delta M(t) + f m_a(t)$$  \hspace{1cm} (3.12)
Equating Eq. 3.12 with Eq. 3.3 we have $a + b = -1/T_{1\text{app}}$. From the definition of $T_1$, we know that $m(t) = \exp(-t/T_1)$ for a single well-mixed compartment of longitudinal relaxation $T_1$. Hence $a = -1/T_1$ giving $b = -f/\lambda$ and $r(t) = \exp(-ft/\lambda)$. Using this form for the residue function is therefore equivalent to using $m_r(t) = M(t)/\lambda$ in the Bloch formulation and therefore assumes immediate equilibration of blood water and extravascular water molecules.

Progress has been made in extending both types of model to include the effects of delayed blood arrival time [4], magnetisation transfer [137], arterial and venous compartments [189, 4] and restricted vessel permeability [189, 237, 184, 52, 234].

### 3.2 Correction for restricted vessel permeability

In reality the labelled blood water does not exchange immediately with the extravascular water so using a single well-mixed compartment is inaccurate. This leads to two opposing effects when measuring perfusion.

Firstly, if labelled water molecules remain in the blood for some time before entering the extravascular space they will relax with the longer blood relaxation time, $T_{1b}$, which is measured as 1.4 s [170] compared to extravascular $T_1$, $T_{1e}$ which could be as low as 0.6 s in white matter [188]. The signal decay will be slower, and the tissue magnetisation larger than predicted by the single compartment model. I call this the $T_1$ effect. The application of diffusion gradients which aim to crush the intravascular signal have little effect in such small vessels [58] and the signal we measure is from both capillaries and tissue.

Secondly, some of the labelled blood water molecules will pass directly through the microvasculature without ever exchanging. The venous magnetisation $\Delta m_v$ will be larger, and the tissue magnetisation lower than predicted by the single compartment model. I call this the outflow effect.

A complex interplay of flow rate, field strength (which affects $T_{1b}$ and $T_{1e}$) and tissue characteristics will determine which effect is dominant, and hence whether perfusion is over or under-estimated when ignoring these effects.
There have been three main approaches in extending the single-compartment model to include the effects of restricted vessel permeability: 1) introducing an extraction fraction $E$ which describes the fraction of water extracted into the extravascular space during a single capillary transit; 2) Introducing an exchange time at which labelled molecules cross from the blood into the extravascular space; 3) Using the permeability surface area product, PS, of the capillary wall to water to describe the rate of water movement between the blood and extravascular compartments. I will describe these advances in turn.

3.2.0.1 Extraction fraction, $E$

Earlier work on restricted vessel permeability [184] describes the effect in terms of a reduction in the water extraction fraction, $E$, during a single capillary transit. A single well-mixed tissue compartment is assumed and $E$ is introduced as a correction factor. More recent work [183, 234] estimates $E$ by sensitising the signal to either blood or extravascular water. The first study by Silva et al. [184] used diffusion gradients to separate the signal contribution from slow (water in the extravascular space) and fast (blood water) diffusing molecules. The second study by Zaharchuk et al. [234] used an intravascular contrast agent to increase the transverse relaxation rate of blood, hence removing the signal from blood water protons. Both studies estimated an extraction fraction of less than one, which decreased with increasing perfusion.

3.2.0.2 Exchange time, $T_{ex}$

Perhaps the simplest approach, and the one I first investigated, is to extend the Bolus model equations to include a time of exchange $T_{ex}$ [158]. For $t<T_{ex}$, $m(t) = \exp(-t/T_{1b})$ and for $t \geq T_{ex}$, $m(t) = \exp(-t/T_{1e})$, i.e. before exchange the water molecules remain in the blood and relax with the $T_1$ of blood. After exchange they are in the extravascular space and relax with the $T_1$ of extravascular space, $T_{1e}$. If a single exchange time is used at which all of the molecules instantaneously exchange, there is a discontinuity in the signal curve at $t=T_{ex}$. For a realistic model, a distribution of exchange times must be used. There are few published values of mean exchange times and they vary considerably from 0.6 to 2 s [158, 221]. The widely used model of Alsop et al.[4] takes a similar approach but using the Bloch equations. Within the voxel there is an arterial blood compartment in addition to the well-mixed tissue compartment with exchange
between the two compartments occurring at a fixed time.

### 3.2.0.3 Permeability surface area product, PS

More recent work includes restricted permeability in the model through the use of PS, the permeability surface area product. The work of Zhou *et al.* [237], based on a model by Schwarzbauer *et al.* [179], extends the Bloch equation model to include both a blood and an extravascular compartment. It is assumed that the blood compartment is well-mixed, i.e. as soon as water enters the blood compartment it instantaneously equilibrates throughout the compartment. His work shows that at high field strengths in an animal model, the current single compartment model will underestimate perfusion. Two other studies [189, 52] do not assume a well-mixed blood compartment, allowing the blood signal to change with both distance and time. The work by St Lawrence *et al.* [189] extends the bolus model to include separate terms in the residue function for blood and extravascular compartments. The model is extended further to include a venous compartment. The model predicts an overestimation of perfusion at low perfusion rates and an underestimation at high perfusion if the current single compartment model is used. The work by Ewing *et al.* [52] describes the changes to the model in terms of changing the effective $T_1$ of the experiment. For flow rates up to 150 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$ at 7T his work shows that the single compartment model will overestimate perfusion.

Much of this previous work has been theoretical with little validation of the models. The aim of my work is to use a relatively simple model with few free parameters in order that it can be tested with *in vivo* data. I extend the Bloch equation model to include exchange between the blood and extra-vascular compartments in terms of PS. It is assumed that the labelled blood water enters the voxel in exchanging vessels, and also leaves through exchanging vessels (i.e. there is no arterial or venous compartment). As Ewing *et al.* [52] notes this can include vessels other than the classic definition of a capillary, for example post-capillary venules. A second assumption is that the blood compartment is well-mixed, which forces assumptions to be made about the venous magnetisation flowing out of the voxel. However, these assumptions may be valid in certain systems (for example human perfusion rates at 1.5T, with $\Delta m_r=0$), and allow for further simplification of the model solution. This enables the model to be tested on *in vivo* data, as described in Chapter 4.
3.2.1 A two-compartment model

A two-compartment system is used with a blood water compartment and an extravascular water compartment, each with corresponding volumes and longitudinal relaxation times, separated by semi-permeable endothelium as shown in figure 3.3.

![Diagram of the two-compartment model](image)

Figure 3.3: Schematic diagram of the two-compartment model. Parameter definitions are given in Table 3.1.

Equation 3.1 can be extended to include permeability and applied to each compartment. The total magnetisation in the difference image will then be the sum of the magnetisation from each compartment multiplied by the relative volumes of the compartments:

\[
\Delta M(t) = v_{ew} \Delta m_e(t) + v_{bw} \Delta m_b(t)
\]  

(3.13)

\(v_{ew}\) and \(v_{bw}\) are the respective volumes of extravascular and blood water per unit tissue volume and \(m_e\) and \(m_b\) are the magnetisations of water in the extravascular water and blood water spaces respectively. Although an improvement to the single compartment model this two-compartment model is not completely accurate since both blood water and extravascular water compartments are assumed to be well mixed. In reality there will be a concentration gradient of labelled water molecules along the capillary from the arterial to the venous end.

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The blood compartment

The blood compartment includes all of the blood in the voxel which could contain labelled water molecules, including arterioles and venules supplying and draining the capillary beds within the voxel. Equation 3.14 below describes the change in magnetisation of the blood compartment, \( m_b(t) \) with time.

\[
\frac{d(v_{bw}m_b(t))}{dt} = \frac{v_{bw}m_e^0 - v_{bw}m_b(t)}{T1_b} + f m_a(t) - f m_e(t) + PS(m_e(t) - m_b(t)) \tag{3.14}
\]

It is a modified version of Eq. 3.1. Magnetisations \( m_b \) and \( m_e \) are represented by lower case letters since they refer to the magnetisation per unit volume of water within a given compartment. Blood water volume \( v_{bw} \) (i.e. blood water volume per unit volume of tissue) is used so that every term is expressed in units of magnetisation per unit volume of tissue per unit time. \( v_{bw} \) is the product of \( v_b \), the volume of blood per unit volume of tissue and \( v_w^b \), the volume of water per unit volume of blood: \( v_{bw} = v_b v_w^b \). \( M^0 \) is replaced by the equilibrium magnetisation of blood water, \( m_e^0 \) and \( T_1 \) by \( T1_b \). The final term describes the flow of labelled water protons between the two compartments. This is normally expressed as \( PS \) multiplied by the difference in tracer concentration between the two compartments. The difference in magnetisation \( m_e(t) - m_b(t) \) is equivalent since this is proportional to the concentration of labelled water molecules or ‘tracer’ in the water that can move between the two compartments.

The extravascular compartment

This equation is simpler than that for the blood compartment since there is no net inflow or outflow of extravascular spins. The only entry of labelled molecules is through the permeable capillary wall.

\[
\frac{d(v_{ew}m_e(t))}{dt} = \frac{v_{ew}m_e^0 - v_{ew}m_e(t)}{T1_e} + PS(m_b(t) - m_e(t)) \tag{3.15}
\]

Here \( v_{ew} \) is the product of \( v_e \), the volume of extravascular space per unit volume of tissue and \( v_w^e \), the volume of water per unit volume of extravascular space: \( v_{ew} = v_e v_w^e \). \( v_e = 1-v_b \) and \( \lambda = \frac{m_e^b + m_w^b}{m_w^e} \) can be used to calculate \( v_{ew} \). These equations are similar to those of Zhou [237] and Schwarzbauer [179] but with \( PS \) stated explicitly from the start. The model is described in terms of the extravascular water and blood water volumes with the blood brain partition coefficient, \( \lambda \) used only to link these two quantities. The compartments are defined as water compartments such that \( m_b(t) \) and \( m_e(t) \) are per unit volume of water. At equilibrium we have from Eq. 3.15: \( PSm_e^0 = PSm_b^0 \), since
\[
d(ve\text{e}m_e(t))/dt = 0 \quad \text{and} \quad ve\text{e}m_e(t) = ve\text{e}m_e^0.
\]
This gives the correct result \(m_b^0 = m_e^0\) with labelled water molecules equally distributed throughout the accessible water.

Assuming that the physical constants \(v_{bw}, v_{ew}, T_{1b}, T_{1e}, m_b^0, m_e^0, f\) and PS do not change between the labelled and control scans we have from 3.14 and 3.15:

\[
d(ve\text{bw}Am_b(t))/dt = \frac{-v_{bw}\Delta m_b(t)}{T_{1a}} + f\Delta m_a(t) - f\Delta m_e(t) + PS(\Delta m_e(t) - \Delta m_b(t)) \quad (3.16)
\]

\[
d(ve\text{e}Am_e(t))/dt = -\frac{v_{ee}\Delta m_e(t)}{T_{1e}} + PS(\Delta m_b(t) - \Delta m_e(t)) \quad (3.17)
\]

### 3.2.2 Solutions to the two-compartment model

Equations 3.16 and 3.17 can be solved to find \(m_b\) and \(m_e\) and hence the total magnetisation using Eq. 3.13 provided we know \(Am\). \(Am\) can be calculated dependent on the labelling strategy. However the assumption that \(\Delta m_e = \Delta M/\lambda\) as for the single compartment theory cannot be made since we are allowing for the possibility that equilibrium between blood and extravascular water molecules is not reached.

It may seem natural to assume that the venous blood has the same magnetisation as the voxel blood compartment. However this will overestimate the venous magnetisation since at short times after labelling the outflow of labelled molecules will be small as they have not yet had time to travel through the vasculature. For a precise definition of \(\Delta m_e\) the distribution of labelled molecules with distance along the vessels within the voxel must be considered [189]. In truth, a compartmental model can never deal with this situation properly and assumptions must be made. However, in certain conditions reasonable assumptions can be made about \(\Delta m_e\) that will simplify the model solutions.

#### The slow solution

We can assume that the labelled water never leaves the tissue voxel during the measurement time, thus giving zero venous magnetisation, i.e. \(\Delta m_e = 0\). For a typical human system the mean transit time (MTT) of labelled molecules (MTT=\(v_b/f \approx 0.05/0.01 = 5\) s) is longer than the measurement time (typically 1 s for FAIR, 3 s for CASL) so this assumption is valid. I call this the slow solution because it is more accurate at slow perfusion rates. St. Lawrence's model [189] supports this assumption, showing that the outflow effect is negligible for typical human flow rates.
The fast solution

At the other extreme of fast perfusion we can assume the venous blood has the same magnetisation as blood in the voxel with \( \Delta m_w = \Delta m_b v^b_w \). For a higher flow animal system the MTT is shorter than the measurement time (MTT \( \approx 0.05/0.05 = 1 \) s) so this assumption is valid. I call this the fast solution because it is more accurate at fast perfusion rates.

The distributed solution

For a complete solution we can set \( \Delta m_a = 0 \) for \( t < \text{MTT} \) and \( \Delta m_a = \Delta m_b v^b_w \) for \( t > \text{MTT} \). However this introduces a discontinuity in the signal curve at \( t = \text{MTT} \). To smooth the transition around MTT a distribution of transit times centred on MTT can be used. Each transit time will produce a slightly different signal curve, which is averaged to give the final signal curve. This is equivalent to a range of perfusion rates producing an average perfusion \( f \). I call this the distributed solution. In this model I use a Gaussian distribution of perfusion rates with full width at half maximum of \( 0.7f \). This width was chosen to be the minimum width required to adequately smooth the signal curve. Further work could look at the use of more realistic flow distributions.

Using these boundary conditions on \( \Delta m_a \) and \( \Delta m_w \) for all three models the coupled differential equations (3.16 and 3.17) were solved manually by means of Laplace transforms [24]. Mathematica [218] was used to calculate the individual transforms.

FAIR

For the pulsed labelling technique of FAIR [102] I use \( \Delta m_a = 2m^0_a \alpha (\exp(-t/T_{1b}) \) for \( t \geq 0 \) and \( \Delta m_a = 0 \) for \( t < 0 \) giving solutions:

\[
\Delta M(t) = \frac{\Delta M(t)}{E} \left[ \frac{(G-D+A)e^{-Dt}}{(D-J)(D-G)-AB} + \frac{(G-J+H+2A)e^{\frac{1}{2}(H-J-G)H}}{H(2D-J-G+H)} - \frac{(J-G+H-2A)e^{-\frac{1}{2}(J+G+H)H}}{H(J+G+H-2D)} \right] (3.18)
\]

Where \( A = PS/v_{bw} \), \( B = PS/v_{sw} \), \( C = 1/T_{1x} \), \( D = 1/T_{1b} \), \( E = 2f \alpha m^0_a \), \( G = B + C \), \( H = (4AB + (J - G)^2)^{\frac{1}{2}} \).

Distributed solution: \( J = A + D \) for \( t < \text{MTT} \) and \( J = A + D + f/v_b \) for \( t \geq \text{MTT} \). Distribution of transit times centred on MTT.

slow solution: \( J = A + D \).
fast solution: \( J = A + D + f/v_b \).

**CASL**

Here I consider the solution for the rising part of the curve only, before the trail end of the labelled bolus reaches the imaging slice. I use \( \Delta m_a = 2m_0\alpha(\exp(-t_A/T1) \) for \( t \geq t_A \) and \( \Delta m_a = 0 \) for \( t < t_A \) where \( t_A \), the arrival time, is the time taken for the labelled blood to travel from the labelling plane to the tissue. I find solutions:

\[
\begin{align*}
\Delta M(t) &= 0 \quad (t < t_A) \\
\Delta M(t) &= E e^{-D(t-A)} e^{-\frac{1}{2}(J+G+H)t'} \\
&\quad \frac{2H(AB - JG)}{[(e^{H't'} - 1)(G^2 - GJ + A(2B + J + G)) + H(G + A)(1 + e^{H't'} - 2e^{\frac{1}{2}(J+G+H)t'})]} \\
&\quad \quad (t_A \leq t \leq t_A + t_L)
\end{align*}
\]

(3.19)

(3.20)

Where \( t' = t - t_A \).

I was unable to solve these equations analytically for part 3 of the CASL curve when \( t > t_L + t_A \) (Fig. 1).

Parameters from Table 3.2 for grey matter at 1.5T were used in the models to plot

![Diagram](image)

**Figure 3.4:** Solutions of the two-compartment model. The distributed solution smooths the transition between the fast and slow solutions. Plotted using parameters for grey matter at 1.5T from Table 3.2.

the forms of the slow, fast and distributed models, figure 3.4. It can be seen that
the distributed model smooths the transition between the two extremes of fast and slow. The tightness of this transition is determined by the width of the transit time distribution. The figures show that for FAIR with typical measurement times of 1 s the slow solution will be accurate, whereas for CASL with measurement time around 3 s neither the slow or fast assumptions will be entirely accurate.

3.2.2.1 Simplified solutions neglecting backflow

One further general assumption is to neglect backflow of labelled water molecules from the extravascular compartment into the blood. That is to assume that the magnetisation in the extravascular water compartment is much less than that of the blood water compartment, i.e. \( \Delta m_e \ll \Delta m_b \). This relies on the fact that the extravascular water volume is much larger than the blood water volume (typically 20:1) and measurement times are short such that \( \Delta m_e \) is far from equilibrium. Justification for this assumption is given in section 3.2.5.3. This is a very useful simplification since it decouples 3.16 and 3.17 producing simpler solutions. This is particularly useful in the case of CASL since it enables solutions for the complete signal curve, including part 3, to be found.

I use the same constants and the same expressions for \( \Delta m_a \) as in the previous section with the addition of \( t_L \), the duration of labelling used in continuous labelling such that \( \Delta m_a = 0 \) for \( t > t_A + t_L \).

**FAIR**

\[
\Delta M(t) = 2f m_0^2 \alpha \left[ \frac{e^{-Dt} - e^{-Ct}}{C - D} - \frac{e^{-Jt} - e^{-Ct}}{C - J} + \frac{1}{A} \left( e^{-Dt} - e^{-Jt} \right) \right]
\]

(3.21)

**CASL**

\[
\Delta M(t) = \begin{cases} 
0 & (t < t_A) \\
2f m_0^2 \alpha e^{-Dt_A} \left[ \frac{1 - e^{-Jt'}}{J} + A \left( \frac{J - C + Ce^{-Jt'} - Je^{-Ct'}}{JC(J - C)} \right) \right] & (t_A \leq t \leq t_A + t_L) \\
2f m_0^2 \alpha e^{-Dt_A} \left[ \left( \frac{1}{J + \frac{A}{J - C}} \right) (e^{Jt_L} - 1) e^{-Jt'} - \frac{A}{c(J - C)} e^{Ct_L} (e^{Ct_L} - 1) \right] & (t > t_A + t_L) 
\end{cases}
\]

(3.22)

(3.23)

(3.24)

Again, the value of \( J \) distinguishes between the slow, fast and distributed solutions.
This simplification has particular benefits for the slow model giving a solution with only one more free parameter than that of the single compartment theory; this is PS/vbf. This is of particular interest for human imaging at 1.5T where we are likely to be in the slow flow regime. In cases where PS/vbf is unlikely to change it could be pre-set from published data, leaving perfusion as the only free parameter to be determined. Alternatively it is possible to estimate this potentially useful parameter by sampling the signal curve at a number of time points. Since capillary surface area, S and volume, v, both depend on the geometry of the vessels, a simple model with vessels of uniform radius r shows that PS/vbf reduces to 2P/vbf r. vbf is a global parameter that could be measured from a blood sample and is simply related to haematocrit. Disease could change either permeability P, vessel radius r or blood water content vbf making PS/vbf a useful measurement in a number of applications. In multiple sclerosis an increase could indicate early damage to the blood brain barrier causing an increase in water permeability. During neuronal activation a decrease could indicate vessel dilation. Changes would also be expected in stroke and tumours.

3.2.2.2 The impermeable solution

It is possible that a model with a single blood compartment (PS=0, impermeable) is more accurate than a single tissue compartment (PS=∞). This solution assumes that the measurement is made before any exchange takes place and therefore also before any labelled protons flow out of the voxel. Hence we can assume that the outflow signal is zero and Δm=0. The impermeable solution was found using equation 3.1 with T replaced by T1, and Δm=0. Using the same expressions for Δm as in the previous sections gives:

**FAIR**

\[ \Delta M(t) = 2f m_0^0 \alpha t e^{-\frac{t}{T_1}} \]  

(3.25)

**CASL**

\[ \Delta M(t) = 0 \quad (t < t_A) \]  

(3.26)

\[ \Delta M(t) = 2f m_0^0 \alpha T_1 (e^{-\frac{t}{T_1}} - e^{-\frac{t}{T_2}}) \quad (t_A \leq t \leq t_A + t_L) \]  

(3.27)

\[ \Delta M(t) = 2f m_0^0 \alpha T_1 e^{-\frac{t}{T_1}} (e^{-\frac{t}{T_2}} - 1) \quad (t > t_A + t_L) \]  

(3.28)
Note that these equations are the same as for the single compartment model Eq. 3.8 with $T_{1,app}$ replaced by $T_{1b}$. The solution has fewer free parameters than the original single compartment with no need for a tissue $T_1$ measurement. For part 3 of the CASL curve (Fig 1) when $t \geq t_A + t_L$, Eq. 3.28, the solution is also independent of blood arrival time $t_A$.

### 3.2.3 Testing the two-compartment model

The solutions to Eq. 3.16 and Eq. 3.17 are complex and there is always a possibility of error. They were tested in two ways: First, it was verified that as $PS$ reaches infinity the solutions reduce to the single compartment solution as shown in figure 3.5. Parameters for human grey matter at 1.5 T from Table 3.2 were used in the distributed form of Eq. 3.18.

![Figure 3.5](image)

**Figure 3.5:** The distributed solution with increasing capillary permeability. As $PS$ increases the solution reduces to that of the single compartment model.

This is to be expected since if we sum equation 3.16 and equation 3.17 the permeability terms cancel and we are left with equation 3.3 for the single compartment case with:

$$\frac{1}{T_1} = \frac{v_{bw}}{v_{bw} + v_{bw}} \left[ \frac{1}{T_{1b}} \right] + \frac{v_{ew}}{v_{ew} + v_{bw}} \left[ \frac{1}{T_{1e}} \right]$$

(3.29)

i.e. the tissue relaxation rate is a weighted sum of contributions from water in blood and extravascular spaces. To check for mathematical errors the solutions were successfully substituted back in to the differential equations.
3.2.4 Evidence for restricted capillary wall permeability

Before comparing simulations of the model solutions we need to find parameter values to use in the simulations, including a value for PS.

PS has been measured by a number of different methods in a number of species [234, 51, 64, 193, 68]. Published values in whole human brain vary from 0.9 to 1.7 min\(^{-1}\) with a mean value of 1.2 min\(^{-1}\) [160, 61, 86]. Two of the studies [160, 61] sampled the outflow of venous blood after intracarotid injection of tritiated water to determine the extraction fraction of labelled water and from that calculate PS. Tritiated water is heavier than normal water, and could have a slightly different PS. The more recent study by Herscovitch [86] compared the measured perfusion values from PET when using either \(^{11}\)C butanol or \(^{18}\)O water as the tracer. Butanol is more permeable than water since it can travel through the lipid layer in the capillary wall. The differences in the perfusion estimates can be attributed to the lower PS of water than butanol. PS values for water also vary within the brain from 0.8 min\(^{-1}\) in white matter to 1.5 min\(^{-1}\) in central cortex [86], thought to be due to different capillary densities producing differences in surface area S, rather than changes to vessel permeability, P. Other studies [184] have shown that the water extraction fraction during a single capillary transit is less than 1; equivalent to a finite PS. Outside the brain, capillary PS values for water are typically a magnitude larger. One study in the rat measured PS of cerebral cortex as 3.3 ± 0.2 and tongue muscle as 14.1 ± 2.5 ml min\(^{-1}\) g\(^{-1}\) [179], supporting the idea that the blood brain barrier is a major barrier to water transport. These studies have firmly established that the capillary wall has limited permeability to water. In my simulations I use the values of Herscovitch [86].

3.2.5 Accuracy of the simplified solutions

Typical parameters for human grey matter at 1.5T, as shown in Table 3.2, were used to plot the form of the distributed solutions \(m_e\) and \(m_b\) as shown in Fig 3.6. They have been multiplied by the relative volumetric fractions such that they represent magnetisation per unit volume of tissue. It can be seen that \(m_b\) peaks before \(m_e\) which is to be expected as the bolus moves through the blood compartment into the extravascular compartment. The average timing of these peaks can be related to the time of exchange, \(T_{ex}\) which was introduced in my initial, crude two-compartment model [158].
If we assume that the distributed solution is an accurate model of the true system we can use it to test the accuracy of the simplified solutions, including the original single compartment solution. The distributed solution was used to produce simulated data at a range of perfusion rates for six systems with parameters as shown in Table 3.2: human grey matter (GM) and white matter (WM) at 1.5T, 4T and 7T. The original single compartment solution, the simplified slow and fast solutions (neglecting backflow) and the impermeable solution were used to estimate perfusion values from the data at time points of t=T1 for FAIR and t=3 s for CASL, typical measurement times that would be used in practice. The perfusion values were compared to the original values used to generate the data.

3.2.5.1 The single compartment model

Figures 3.7 a-d. show the errors of the single compartment model for both pulsed and continuous labelling at a range of field strengths and perfusion rates. At low perfusion rates the single compartment theory overestimates perfusion due to the dominance of the $T_1$ effect while at higher perfusion rates the outflow effect begins to dominate and perfusion is underestimated. At higher fields this switching between the two effects
Figure 3.7: Errors of the single compartment theory at a range of field strengths, tissue types and measurement techniques, using the parameters of Table 2. At low perfusion rates the single compartment model overestimates perfusion due to the $T_1$ effect and at high perfusion rates perfusion is underestimated due to the outflow effect. The transition point and magnitude of the error depend on field strength, tissue type and measurement technique.

occurs at lower perfusion rates due to the greater weighting of the outflow effect as out-flowing spins carry higher magnetisation due to slower decay. Comparing Fig. 3.7c with 3.7a and 3.7d with 3.7b it can be seen that in white matter the $T_1$ effect is larger due to the greater difference between $T_{1b}$ and $T_{1e}$. Comparing Fig. 3.7a with 3.7b and 3.7c with 3.7d it can be seen how the timing of the measurement affects the interplay of the two effects since the FAIR measurement is taken at an earlier time point ($t \approx 1\text{ sec}$) than the CASL measurement ($t \approx 3\text{ secs}$). For the later CASL measurement the outflow effect begins to dominate at a lower perfusion rate because the measurement time is closer to the MTT and un-exchanged molecules will begin to exit the voxel. The effect
of field strength is to change the $T_1$ values of the blood and tissue which in turn determine the strength and relative importance of the venous magnetisation, $\Delta m_v$. At higher fields $\Delta m_v$ increases and the outflow effect begins to dominate over the $T_1$ effect at a lower perfusion rate. The distribution of transit times will determine the smoothness of transition between these two effects. For typical human perfusion rates at 1.5T (see Table 3.2) I find that for CASL the single compartment theory overestimates perfusion by 17% in grey matter and 62% in white matter. This error is strongly dependent on the blood $T_1$, $T_1^\beta$.

The work by St. Lawrence et al. [189] shows almost identical results for their single-pass approximation model, without the assumption of a well-mixed blood compartment. This helps to verify my handling of the venous outflow ($\Delta m_v=0$) to correct for the assumption of a well-mixed blood compartment. Interestingly, the inclusion of a venous compartment in the St. Lawrence model significantly changes the simulation results, reducing the outflow effect. It is therefore important to establish if this venous compartment is required. The work by Zhou et al. [237] does not show an overestimation by the single compartment model at low perfusion rates. There are two possible reasons for this discrepancy. First, Zhou et al. assumed a smaller value of $T_1^\beta$ which will reduce the $T_1$ effect. Second, the model used did not take account of the overestimation of venous outflow that results from assuming a well-mixed blood compartment. This will overemphasize the outflow effect, and therefore mask the $T_1$ effect. Ewing et al. [52] predict the crossover between the two effects to occur around 150 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$ at 7T. From Fig. 3.7a I find a similar value of 130 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$.

In addition I find that the balance of the $T_1$ effect and the outflow effect depends not only on perfusion and relaxation parameters but also on the measurement technique and the measurement time. FAIR is more sensitive to the $T_1$ effect because measurements are made about 1 second after labelling when there is negligible outflow of labelled protons. The $T_1$ effect dominates up to flow rates in excess of 200 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$ at 1.5T. CASL imaging is typically around 3 seconds after the start of labelling by which time there is some outflow of labelled protons. The outflow effect dominates for flow rates in excess of 100 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$ at 1.5T. In either case, for human scanning at 1.5T it is the $T_1$ effect that dominates and the single
compartment model will overestimate perfusion. During human neuronal activation it is likely that the increased perfusion will change the balance of these two effects. It is important to consider what effect this will have on the analysis of functional imaging studies. This is considered in Chapter 6. Note that the simulations assumed zero arrival time. An increased arrival time would tend to delay the outflow effects, causing the $T_1$ effect to dominate for a wider range of perfusion rates.

3.2.5.2 The impermeable solution

![Graphs showing errors of the impermeable solution at a range of perfusion rates for different tissue types and measurement techniques.](image)

Figure 3.8: Errors of the impermeable solution at a range of perfusion rates for different tissue types and measurement techniques. There was found to be no dependence on field strength. Perfusion is always underestimated.

The impermeable solution simplifies the model even further to a single blood compartment. Figure 3.8 shows the errors of the impermeable solution for both pulsed and continuous labelling at a range of perfusion rates. In all cases the impermeable solution underestimates perfusion. There was found to be no dependence on field strength. FAIR showed an apparent increase in error with increasing field strength but this was due to the change in measurement time ($t = T_{1e}$). All of the curves for FAIR correspond to a measurement time of 1 s. The errors for FAIR are much smaller than for CASL, on the order of 3% for grey matter and 7% for white matter. This is because the measurement is taken at 1 s rather than 3 s and the proportion of protons that have
crossed into the extravascular space is small. The errors are larger for white matter than grey matter because the difference in $T_1$ between blood and extravascular space is larger. For FAIR the impermeable model is more accurate than the original single compartment model and a tissue $T_1$ map is no longer required for quantification. This is particularly attractive since it will reduce the scan time and the need for registration of the perfusion images to a $T_1$ map. This model is clearly a good option for FAIR modelling. It may be advantageous to reduce the CASL labelling time and delay time to give a shorter measurement time (2 s is feasible) in order to improve the accuracy of the impermeable model.

The only free parameters of the impermeable model are perfusion $f$, $T_{1b}$ and $t_A$, being independent of $T_{1e}$. For part 3 of the CASL curve equation 3.28 (see fig 2.7 from chapter 2) or FAIR the model is also independent of $t_A$. It could be possible therefore to use this model as a method for measuring $T_{1b}$. For FAIR the maximum of the signal curve is found at $t=T_{1b}$.

### 3.2.5.3 The simplified slow and fast solutions

We need to examine the accuracy of both the assumption that $\Delta m_e \ll \Delta m_b$ and the slow and fast assumptions, i.e. $\Delta m_e = 0$ and $\Delta m_e = \Delta m_b v_w^b$.

First, to consider the accuracy of neglecting backflow I compare the solutions with and without this assumption, i.e. comparing equations 3.18 and 3.19 (both fast and slow equations) to equations 3.21 and 3.23. The general solutions (equations 3.18 and 3.19) were used to produce simulated data at a range of perfusion rates and field strengths using parameters of Table 3.2. The simplified solutions were used to estimate perfusion values from the data at time points of $t=T_{1e}$ for FAIR and $t=3$ s for CASL.

Figure 3.9 shows the error in the perfusion estimate when neglecting backflow for the case of grey matter at 1.5 T. The errors are extremely small, never rising above 0.6% regardless of field strength or tissue type. The changes in error with perfusion are however of interest and help us to understand the dynamics of the system. For the slow solution the assumption means the neglect of a proportion of labelled protons that flow back into the vasculature – this proportion is independent of perfusion. The magnetisation of these protons will relax more slowly in the blood and neglect of them will
cause an underestimation of signal and therefore an overestimation of perfusion. For the fast solution we see the conflicting effect of these backflowing protons flowing out of the voxel at higher perfusion rates. A neglect in this case will cause an overestimation of signal and therefore an underestimation of perfusion.

This assumption is negligible and so we can move on to take both assumptions together (i.e. $\Delta m_e \ll \Delta m_b$ and $\Delta m_e = 0$ or $\Delta m_e = \Delta m_b v_b^0$) and determine the accuracy of the slow and fast simplifications. Using the same methods as described at the beginning of this section the simplified slow and fast solutions were used to calculate perfusion from data generated by the general distributed solution.

Figure 3.10a-d shows the accuracy of the simplified fast and slow two-compartment solutions at 1.5T. It can be seen that the slow solution is very accurate for the FAIR technique at typical human perfusion rates in both grey and white matter, being almost indistinguishable from the distributed solution. With CASL the slow solution becomes inaccurate at lower perfusion rates than for FAIR because of the later measurement times that approach MTT. When the MTT is reached the outflow effect, which is assumed negligible in the slow solution, becomes important. The fast solution is only accurate for higher perfusion rates with the outflow effect being overestimated at slower rates. The slow solution is therefore fairly accurate for human studies at 1.5T where perfusion rates are typically below 80 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$. This solution
Figure 3.10: Errors of the simplified two-compartment solutions at 1.5T for different tissue types and measurement techniques using parameters of Table 3.2. The slow solution is accurate in both tissue types for FAIR but becomes inaccurate for higher perfusion when using CASL. The fast solution is only accurate at perfusion rates above 100 ml blood min⁻¹ (100 ml tissue)⁻¹ for CASL and higher perfusion rates for FAIR.

is attractive since it has only one extra free parameter, PS/vbw, than the original single compartment solution. Two options are then possible, either assume a value for PS/vbw or fit for it as an extra free parameter. Which of these to choose depends on two main factors: the SNR of the data and the application. If PS/vbw is likely to vary across the brain due for example to an infarct then it is wise to fit for PS/vbw or perfusion estimates could be inaccurate. If however it can be assumed to be stable then it would be better to assume a value for PS/vbw and thus obtain a more precise fit for perfusion. The errors
of the slow and fast solutions depend on the width of the transit time distribution with a tighter distribution extending the range of their accuracy.

3.2.6 Inaccuracies of the two-compartment model

3.2.6.1 Lack of an arterial or venous compartment

The model assumes that the labelled blood enters and leaves the voxel in exchanging vessels. This is unlikely to be true. First, at the arterial end, some blood in large vessels will flow straight through the voxel and should be excluded from the measurement. However, blood entering in larger arterioles should be included, in fact any blood that will eventually perfuse the measurement voxel. This is discussed in section 3.4. At the venous end blood may exit in non-exchanging venules. St Lawrence et al. [189] have shown that the inclusion of a venous compartment reduces the outflow effect. The inclusion of arterial and venous compartments may improve the accuracy of the model, but would also increase the number of free parameters. Alternatively, in my model, PS can be thought of as an average PS for all of the vessels in the imaging voxel that contain labelled blood water.

3.2.6.2 Assumption of a well-mixed blood compartment

The model falsely assumes a well-mixed blood compartment. The main effect of this is on the outflow effect which is either over- or underestimated depending on the assumptions made for the venous magnetisation $m_v$. However, I have shown that these assumptions may be valid under certain conditions, specifically $\Delta m_v = 0$ for human perfusion at 1.5T and $\Delta m_v = \Delta m_w v_w$ for high perfusion rates at high fields. The simulations of St. Lawrence et al. [189] using a distributed model produce very similar results to my model for human perfusion at 1.5T, which help validate this assumption.

3.2.6.3 Magnetisation transfer effects

One effect not included in the model is the time-varying $T1_e$ with the CASL technique due to the magnetisation transfer effects of the labelling pulse. Although no direct saturation of the water magnetisation in the imaging slice occurs due to the narrow linewidth of the free water peak, saturation of the macromolecular molecules does occur. This results in attenuation of the free water signal via magnetisation transfer, shortening $T1_e$. The effect will diminish with increasing delay time. At zero delay time $T1_e$ will
be close to the saturated value, which for grey matter at 1.5 T is 0.75 s [4]. At longer
delay times $T_1_e$ will recover to its value without MT effects of about 1 s [188, 26]. For
a full treatment of this problem, time-varying $T_1_e$ would have to be included in the
original equations 3.16 and 3.17. This would become prohibitively complex for useful
application but could be interesting future work. As Alsop assumes in his model [4] it is
likely that the MT effects fall off rapidly following the end of labelling. Since only a small
proportion of labelled protons will have spent any appreciable time in the extravascular
space before this time, the effects could be small. The effect of magnetisation transfer
on the blood $T_1$, $T_{1b}$ is negligible [144] at typical offset frequencies and rf amplitudes
used in ASL experiments. The effect of varying $T_1_e$ on estimates of perfusion and
$PS/v_{bw}$ is investigated in Chapter 4.

3.3 Error propagation

The inaccuracies of the many fixed parameters in the model will propagate to pro­
duce errors in the perfusion values. These errors were determined numerically. The
two-compartment slow solution was fitted, by the method of least squares, to the sim­
ulated data of human grey matter at 1.5T using CASL with a slight change to each
fixed parameter in turn. The error in perfusion was calculated by comparing the es­
timated value of perfusion with the value used to generate the data. The magnitude
of the propagated errors will determine the accuracy to which these fixed parameters
must be known if they are not to contribute to the uncertainty of the perfusion estimate.

Figure 3.11 shows the propagated error in the perfusion estimate for each unknown
parameter. While $PS/v_{bw}$ need not be known very accurately, the parameters $T_{1b}$, $m^0_b$
and $\alpha$ must be more accurate since they have a larger effect on the perfusion estimate.
For less than 5% error in perfusion estimates in grey matter (an acceptable limit) the
parameters must be known to the following accuracy: $\alpha$: 5%; $m^0_b$: 5%; $T_{1b}$: 7%; $t_A$:
21%; $T_{1e}$: 27% and $PS/v_{bw}$: 100%. These values represent the accuracy we must strive
for. While the accuracy of the tissue $T_1$ measurement is not too important it is clear
that accuracy of blood parameters $T_{1b}$ and $m^0_b$ are very important - an issue which
has been somewhat overlooked. An accurate measurement of $T_{1b}$ is difficult due to the
movement of the blood. The measurement of $m^0_b$ is discussed in Chapter 4. Note that
the use of $T_{1_{app}}$ rather than $T_1$ in current single compartment theory (see Eq.[4]) is
Error in Perfusion due to fixed parameters

Figure 3.11: Errors in perfusion estimates due to errors in the fixed parameters. Using grey matter parameters at 1.5T from Table 3.2 and the simplified slow solution equation 3.23, measurement time 3 s. The error propagation ratio [197] gives the initial slope of the lines. The blood parameters, $m^b_a$ and $T_{1b}$ and the inversion efficiency $\alpha$ give the largest errors.

insignificant in terms of the accuracy of the perfusion estimate. $T_{1\text{app}}$ is of the order of 1% smaller than $T_1$ which produces only a 0.2% error in perfusion which is negligible when compared to other errors.

Two considerations are 1) whether these fixed parameters will change with position and 2) whether they will cause a linear change in perfusion. For example the labelling efficiency $\alpha$ does not change with position and will cause a linear change in perfusion since the signal change $\Delta M$ is proportional to $\alpha$. If the aim was to differentiate areas of low or high perfusion within the brain the value of $\alpha$ would not matter. However, as soon as we desire absolute values, or wish to compare perfusion values across subjects $\alpha$ will be important. In contrast, $T_{1c}$ does change up to two-fold with position, and so a map is required for accurate perfusion comparison between regions (although the effect may be small for FAIR as described in section 3.2.5.2). $m^g_a$ and $T_{1b}$ do not change
with position. PS/v_{bw} is unlikely to change with position since PS/v_{bw}=2P/r.v_{bw}, where permeability P, vessel radius r and blood water content v_{bw} are unlikely to change with position. Figure 3.11 shows that, at small errors, all of the parameters affect perfusion linearly.

3.4 Correction for many vessel flow

The simulations of the two-compartment model show that for a realistic value of PS a significant proportion of the labelled molecules remain in the vasculature at the time of measurement. From figure 3.6 it can be seen that at a typical measurement time of 1 s for FAIR 74 % of signal originates from the blood compartment. For CASL with a later measurement time of 3 s this proportion is reduced to 62 %. Previous models assuming instantaneous mixing of water have assumed that all of the signal originates from the extravascular compartment.

This distinction becomes important when we consider the removal of unwanted signal from blood in larger vessels. Within a single voxel there are many types of blood vessel, as shown in figure 3.12.

Figure 3.12: The continuum of blood vessel sizes within a single voxel. Taken from MacFall et al. [126].

True perfusion measurements must only include the blood which is actually perfusing the tissue of that voxel. Blood in larger vessels will perfuse tissue downstream
and must be excluded. Models assuming complete exchange of labelled water suggested
that all intravascular signal should be removed. However, as demonstrated in my sim-
ulations, at the time of measurement a large proportion of the labelled water is still in
the blood. Labelled water in any vessel that will lead to a capillary within the tissue
voxel should be included. It should be possible to calculate the diameter of the largest
perfusing vessel as a function of voxel size. Central to these considerations is the need
for an accurate model of the vasculature within a voxel. We need to know the scale of
bifurcations and the size of vessels before we can make any headway on this issue.

Information on this basic anatomical information is surprisingly scarce. There is a
general acceptance that the arterial vessels have fractal characteristics [167, 210] with
similar branching structures at all magnifications as shown in figure 3.13.

Figure 3.13: The fractal nature of the vascular system. At smaller scales the same pattern
of branching is seen. Taken from Henkelman et al. [85].

This implies that the length, \( l_n \), of each vessel segment, \( n \), scales proportionally with
the diameter, \( d \), i.e. \( l_n = C d_n \), for any \( n \), where \( C \) is a constant. This is shown diagram-
atically in figure 3.14. The distance, \( x \), of a feeding vessel from the capillary bed is
simply the sum of \( N \) segment lengths:

\[
x = \sum_{n=0}^{N} l_n
\]  

(3.30)

It has been shown theoretically and verified experimentally that at each branching, \( n \),
the sum of the cubed diameters of the daughter vessels is equal to the cubed diameter
of the parent vessel [85, 173]. Assuming there are 2 branches at each stage the average
Figure 3.14: Branching of the vascular system. The capillary bed is defined as segment $n=0$ with vessel diameter $d_0$ and length $l_0$.

diameter of a parent vessel $d_{n+1}$ in terms of the daughter vessel, $d_n$ is given by:

$$d_{n+1} = d_n \sqrt{2}$$  \hspace{1cm} (3.31)

hence

$$d_n = d_0 2^{n/3}$$  \hspace{1cm} (3.32)

where $d_0$ is the capillary diameter. Since $l_n = C d_n$ it follows that $l_n = l_0 2^{n/3}$. Substituting this into equation 3.30 we have:

$$x = \sum_{n=0}^{N} l_0 2^{n/3}$$ \hspace{1cm} (3.33)

Using capillary diameter, $d_0$, of 10 $\mu$m [135] in equation 3.32 and capillary length, $l_0$, of 200 $\mu$m [200] (and references therein) in equation 3.33 we can calculate $x(n)$ and $d(n)$. We find that vessel diameter increases linearly with distance from the capillary bed as shown in figure 3.15. The values of $d_0$ and $l_0$ affect the gradient of the line.

From figure 3.15 with $d_0 = 10 \mu$m and $l_0 = 200 \mu$m and typical voxel dimensions of 5mm, all vessels up to a diameter of 60 $\mu$m must be included in the perfusion measurement since these could all perfuse the tissue in our voxel. Blood velocity is proportional to vessel diameter [85] so, using capillary velocity of 1mm$s^{-1}$, it follows that we must exclude all blood flowing with a velocity greater than 6mm$s^{-1}$. I call this the critical velocity, $v_{crit}$. This value will scale linearly with voxel dimension. Previous work [58,
Figure 3.15: The increase of vessel diameter with distance from the capillary bed is linear. The gradient depends on capillary diameter \( d_0 \) and capillary length \( l_0 \).

Ye [230] has assumed that we need to include only capillary blood, ignoring perfusing blood that may not have reached the capillaries at the time of measurement.

**Sensitizing the perfusion measurement to different vessel sizes**

There are currently two approaches for removing signal from unwanted vessels. The first idea, developed by Ye [230] is to add a bipolar gradient pair which aim to ‘crush’ the signal from moving arterial water spins. The strength of the ‘b’ value [113] determines the extent of the crushing. In theory if we could crush all of the signal from blood flowing above a certain velocity we could sensitise to the vessel sizes required but the bipolar gradients will cause partial signal loss from all vessel sizes [58] making this a fairly crude tool for quantification.

The second method is to introduce a delay time between the end of labelling and imaging [4]. This gives time for blood in larger vessels to wash straight through the voxel and so be excluded from the measurement. In order to exclude blood flowing above 6\( \text{mm} s^{-1} \) in a 5\( \text{mm} \) voxel we require a delay time, \( t_s \) of about 800 msec (\( 5/6 = 0.8 \) s). I call this the critical delay time, \( \delta_{crit} = \text{voxel size}/v_{crit} \). Perfusion measurements should be insensitive to delay time for values greater than this. Increasing the delay time further will only allow labelled molecules to flow further down the vascular tree into the capillaries, not through the voxel. Interestingly, this critical delay time remains
almost constant with different voxel sizes, assuming the velocity remains linear with di­
ameter over a wide range.

It is possible to predict the dependence of perfusion measurement on delay time
using this model. The velocity above which blood is excluded from the measurement, \( v_{exc} \) is equal to \( \frac{\text{voxel size}}{t_d} \). So the proportion of velocities excluded, \( R \), compared to the total velocity range are:

\[
R = \frac{v_{max} - v_{exc}}{v_{max}} = 1 - \frac{\text{voxel size}}{v_{max} t_d}
\]  

(3.34)

Where \( v_{max} \) is the maximum velocity present in the voxel.

The fractal model predicts that each segment level, \( n \), of figure 3.14 contains an
equal volume of blood [85], i.e. the blood is equally distributed through all vessel di­
ameters. Since velocity is proportional to vessel diameter and vessel diameter to blood
volume it follows that \( R \) is equal to the fraction of blood volume excluded, and \( 1-R \) is
the fraction of blood volume remaining in the voxel. The apparent perfusion measured
will be proportional to the blood volume, i.e. proportional to \( 1-R \). However, when the
delay time reaches the critical delay time, \( \delta_{crit} \), a further increase will cause no more
blood volume to be lost since the labelled blood will simply move further down the
vascular tree within the voxel.

Using ‘perfusion’ as being proportional to \( 1-R \), figure 3.16 shows predicted ‘perfusion’
drop with delay time for a variety of voxel types (i.e. different \( v_{max} \)). It was assumed
that voxel size=5mm, \( \delta_{crit}=0.8s \) and true capillary perfusion=60ml blood min\(^{-1}\) (100
ml tissue\(^{-1}\)).

The vessel types refer to the size of the largest vessel in the voxel and simply change
the maximum blood velocity. For small arterioles the initial increase in delay time will
not change the signal since this is not time enough for blood to leave the voxel through
these vessels. The same inverse law relation is seen for all voxel types. This all assumes
that the labelled blood enters the voxel at delay time of zero. In practice this will be
complicated by different arrival times for different vessel types.
Figure 3.16: Predicted decrease in measured perfusion with increasing delay time, due to the blood in larger vessels flowing quickly through the voxel. For small arteries, \( v_{\text{max}} = 10 \text{cm/s} \), large arteriole \( v_{\text{max}} = 5 \text{cm/s} \), arteriole \( v_{\text{max}} = 2 \text{cm/s} \), and small arterioles \( v_{\text{max}} = 1 \text{cm/s} \).

3.5 Summary

- Correcting for restricted vessel permeability has two opposing effects on the perfusion estimate. Firstly, if labelled water molecules remain in the blood for some time before exchanging the magnetisation will decay more slowly. I call this the \( T_1 \) effect. Secondly, some of the labelled blood water molecules will pass directly through the microvasculature without ever exchanging, increasing the signal decay. I call this the outflow effect.

- Perfusion, field strength, tissue type and measurement time all affect the balance of the two effects. At low perfusion rates the \( T_1 \) effect dominates and at high perfusion rates the outflow effect dominates. At higher field strengths the outflow effect is stronger. White matter is more affected by the \( T_1 \) effect due to the greater difference between blood and tissue \( T_1 \) than in grey matter. At earlier measurement times the \( T_1 \) effect is more dominant.

- At human perfusion rates at 1.5T the assumption that \( \Delta m_w = 0 \) is valid, giving the slow solution with only one extra free parameter than the single compartment model. At high perfusion rates at high fields the assumption that \( \Delta m_w = \Delta m_b v_w^b \)
is valid. For FAIR imaging at 1.5T the impermeable solution is valid, removing the need for a tissue $T_1$ measurement.

- The accuracy of the blood parameters, $T_{1b}$, $m_o^0$ and $\alpha$ are important for an accurate perfusion measurement. Conversely the tissue $T_1$ measurement is less important and may be unnecessary for a FAIR experiment.

- Blood which is present in the imaging voxel, but is not perfusing it should be excluded from the perfusion measurement. A many-vessel model predicts that for CASL imaging a delay time of 0.8s should be adequate to remove this signal.

The results of testing the many-vessel model and the two-compartment model are presented in the next chapter.
<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
<th>Units</th>
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<tbody>
<tr>
<td>f</td>
<td>Blood Perfusion</td>
<td>ml blood/min/100 ml tissue</td>
</tr>
<tr>
<td>PS</td>
<td>Permeability surface area product</td>
<td>ml water/min/100 ml tissue</td>
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<tr>
<td>λ</td>
<td>Brain:blood partition coefficient</td>
<td>ml water/ml tissue (l-1)</td>
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<td>$\lambda = \frac{V_{bw} + V_{bw}}{V_w}$</td>
<td>ml water/ml blood (l-1)</td>
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<tr>
<td>t_A</td>
<td>Arrival time (from labelling plane to image slice)</td>
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<td>Labelling time</td>
<td>s</td>
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<td>t_d</td>
<td>Delay time</td>
<td>s</td>
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<td>G</td>
<td>Gain</td>
<td>signal per unit of magnetisation</td>
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<td>α</td>
<td>Inversion efficiency of labelling pulse</td>
<td>fraction of blood water protons inverted</td>
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<td>M(t)</td>
<td>Tissue magnetisation</td>
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<td>Equilibrium tissue magnetisation</td>
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<td>T₁ₑ</td>
<td>Longitudinal relaxation time of water in extracellular space</td>
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<td></td>
</tr>
<tr>
<td>m(t)</td>
<td>Magnetisation relaxation function. Fraction of magnetisation remaining after decay for time t.</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1: List of Parameters.
<table>
<thead>
<tr>
<th>Field (T)</th>
<th>1.5</th>
<th>4</th>
<th>7</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{1_b}$ (s)$^a$</td>
<td>1.4</td>
<td>1.8</td>
<td>2.3</td>
</tr>
<tr>
<td>Tissue</td>
<td>Grey</td>
<td>White</td>
<td>Grey</td>
</tr>
<tr>
<td>PS (ml water (min)$^{-1}$(ml tissue)$^{-1}$)$^b$</td>
<td>1.5</td>
<td>0.8</td>
<td>1.5</td>
</tr>
<tr>
<td>$T_{1_c}$ (s)$^c$</td>
<td>1.0</td>
<td>0.6</td>
<td>1.35</td>
</tr>
<tr>
<td>$f$ (ml blood (min)$^{-1}$(100ml tissue)$^{-1}$)</td>
<td>60</td>
<td>30</td>
<td>60</td>
</tr>
<tr>
<td>$V_b$ (ml blood (ml tissue)$^{-1}$)$^d$</td>
<td>0.05</td>
<td>0.03</td>
<td>0.05</td>
</tr>
</tbody>
</table>

Table 3.2: Parameter values used for simulations. Also used were $\lambda=0.9$ [169], $\alpha=0.7$ [3], $v_w=0.7$ [146], $t_L=3s$ and $t_A=0$.  

a) 1.5T [170], 7T [12], 4T values interpolated assuming $T_{1_b}$ scales linearly with field strength.  
b) from Herscovitch et al. [86]  
c) 1.5T [188, 206], GM at 4T [103], GM at 7T [12]. Values for WM were interpolated assuming ratio of GM:WM $T_{1_c}$ remains constant.  
d) From Leenders et al. [116].
Chapter 4

Testing the model

The previous chapter put forward a new model for the quantification of perfusion. In this chapter I present the results of testing the model on human ASL data. The new model includes arrival time $t_A$ and the permeability parameter $PS/v_{bw}$ which can be estimated along with perfusion.

4.1 Methods

Measurements were made using the CASL technique on a General Electric Signa 5.6 1.5 T scanner. The code for the pulse sequence was kindly provided by David Alsop [3]. The protocol is as described in figure 2.10 with Gradient Echo, EPI collection.

**EPI parameters**
- Echo time = 34 ms.
- Acquisition bandwidth = 62.5kHz.
- Resolution 64x64 (changed to 32 x 32 for some investigations).
- Field of view 24 cm (giving pixel size 3.75 x 3.75 mm for 64 x 64 resolution).
- Up to 8 slices with variable thickness and gap at least 2mm.
- Slice collection order 1,3,5,7,2,4,6,8 with slice 1 being the inferior slice closest to the labelling plane.
- The time of image collection, $t_i$, relative to the start of the EPI imaging is different for each slice. It is defined as the centre of slice acquisition time for each slice.

**Labelling parameters**
- RF irradiation $B_1=3.5 \times 10^{-6}$ T and labelling gradient $G=2.5$ mT/m. Substituting
these values for $G$ and $B_1$ into the inequalities for adiabatic fast passage (Equation 2.22 of Chapter 2), along with a typical $T_2$ value of 100 ms, I find that the inequalities are satisfied for a velocity range of 1 to 130 cm$^{-1}$. Arterial blood velocities measured using Doppler ultrasound techniques, are in the range of 20 to 70 cm$^{-1}$ [53, 130], falling within the criteria for adiabatic labelling. Macotta et al. [125] have shown, for this gradient strength and rf amplitude, halving or doubling the velocity from 40cm/s only changes the labelling efficiency by 2%.

- The control labelling uses a double inversion with the same average power and centre frequency as the single inversion such that the off-resonance MT effects are matched, as described in [3]. Double inversion is achieved by multiplying the rf irradiation of fixed frequency $f_0$ by a sine wave of frequency $f_1$ to produce irradiation at two frequencies $f_0 + f_1$ and $f_0 - f_1$. The modulation frequency $f_1$ is 125 Hz. The MT matching can be tested by looking for residual subtraction signal on a phantom or when labelling in the air above the head. This is investigated in section 4.2.

- Labelling is pseudo-continuous with blocks of 192 ms with 5 ms gap, maximum of 9 blocks giving labelling duration $t_L = 192.5 \times$ number of labelling blocks, i.e. up to 1732 ms. The gap is required to reduce the duty cycle of the rf amplifiers.

- The labelling plane is usually positioned 4 cm below the inferior slice but this is variable.

- Post labelling delay $t_d$ is variable (typically between 0 and 1.5 s). The measurement time, $t$, is defined as the time of image collection relative to the start of labelling, giving $t = t_L + t_d + t_i$ as shown in figure 4.1. $t$ is different for each slice due to differences in $t_i$.

- Repetition time $T_{RASL} = 4s$ (time between the starts of successive labelling pulses).

- 45 averages give a total scan time of 6 mins (variable). These are averaged to give the final control and labelled images. Future work could look at the co-alignment of the...
individual averages to reduce noise due to motion.

Analysis

Using CASL in humans at a field strength of 1.5T it is reasonable to use the slow solution with the assumption of no back flow as described by equations 3.22, 3.23 and 3.24. Figure 3.10 b and d show that this is predicted to be accurate for grey matter perfusion rates below 80 ml blood min^-1 (100 ml tissue)^-1 and white matter perfusion rates below 40 ml blood min^{-1} (100 ml tissue)^{-1}. Normal human perfusion values as measured by PET fall within this range [116]. From now on I will refer to this solution as the 'two-compartment solution'. This model is compared to the original single compartment model, equations 3.6, 3.7 and 3.8 and the impermeable solution, equations 3.26, 3.27 and 3.28.

The unknown parameters of the two-compartment solution are: the inversion efficiency \( \alpha \), the extravascular and blood relaxation times \( T_{1e} \) and \( T_{1b} \), the equilibrium magnetisation of arterial blood \( m_0^a \), the arrival time \( t_A \), the permeability parameter \( PS/v_{bw} \) and perfusion \( f \). Each of these parameters will be considered in turn.

4.2 Subtraction tests

Before perfusion can be measured it must be established that on subtraction of the labelled and control images there remains no residual signal in the absence of perfusion. Signal could arise if the MT effects of the labelling and control are poorly matched or if the gradients are unstable with poor homogeneity. This can be tested in two ways; by using a static phantom or by applying the labelling above the head where it will label no blood.

Typical examples of these tests are shown in figure 4.2. Over all slices the average signal difference between control and label was 0.01% for the phantom and 0.03% for the head, confirming successful static signal subtraction. It is predicted that the signal difference due to perfusing blood will be on the order of 1% (see figure 3.4) giving errors in perfusion from imperfect static tissue subtraction of around 3%. These results are in agreement with measurements made by David Alsop [3] using the same scanner and technique at a different centre. The explanation for the rings around the edge of
Figure 4.2: Subtraction tests. On the left are shown the average control signal for an axial head slice (top) and a static phantom (bottom). The images on the right show the average subtraction signal (control - label). The images were taken with 1.73 s labelling, zero delay time and 45 averages for the head, 5 averages for the phantom. The control images are shown with 25 times greater signal range than the subtraction images. The head subtraction image is less noisy than the phantom subtraction due to the greater number of averages.

the phantom and head is uncertain but they could be due to magnetic susceptibility effects at the interface between tissue and air. Movement could also contribute to signal differences at the edge of the head. However, more importantly, the subtraction is good inside the head.

4.3 Inversion efficiency

Inversion Efficiency $\alpha$ describes the degree of labelling of the arterial spins and is defined as:

$$\alpha = \frac{m_a^{\text{control}} - m_a^{\text{label}}}{2m_a^0} \quad (4.1)$$

Where $m_a^{\text{control}}$ is the arterial magnetisation following the control inversion, $m_a^{\text{label}}$ is the arterial magnetisation following the labelling inversion, and $m_a^0$ is the equilibrium magnetisation of arterial blood.
4.3.1 Efficiency of the labelling pulse

For perfect inversion $n_{\text{label}}^0 = -n_0^0$. In reality $n_{\text{label}}^0$ will be less negative than this as the blood velocity reaches the boundaries of the adiabatic inequalities, equation 2.22, or when vessels are non-perpendicular to the labelling plane. The efficiency of the single labelling pulse can be found by imaging the arteries downstream of the imaging plane using a flow-compensated imaging technique [236]. Over a wide range of blood velocities, the labelling efficiency is greater than 90% [125].

4.3.2 Efficiency of the control

For perfect restoration of the magnetisation following the double control inversion, $m_{\text{control}}^0 = m_0^0$. In reality, $m_{\text{control}}^0$ will be less than this for the same reasons as the single inversion, except the errors are multiplied. The efficiency of the double control inversion can be found by comparing the difference signal (control - label) in a single tissue slice when using 1) A single inversion pulse with the control labelling placed symmetrically equidistant above the head (giving $m_{\text{control}}^0 = m_0^0$) and 2) Using the double inversion control pulse.

The published results of these measurements from another centre using our labelling parameters of $B_1$ of $3.5 \times 10^{-6}$ T, $G=2.5$ mT/m and modulation frequency of 125 Hz give an average labelling efficiency of $\alpha=0.7$ [3]. I felt that there was no need to repeat these measurements since my pulse sequence and scanner were the same as those used. I use $\alpha = 0.7$ in all measurements.

The value of $\alpha$ will be different for each subject due to differences in blood velocity and vessel architecture. Assuming the same value of $\alpha$ for all subjects will introduce an error in the absolute perfusion value. Since inversion efficiency is global (i.e. the same across the brain), relative perfusion measurements within the brain will not be affected.

4.4 Blood $T_1$

The error propagation analysis (figure 3.11) showed that for an accurate perfusion measurement the blood $T_1$, $T1_b$, is an important measurement. Unfortunately, blood $T_1$ is
hard to measure in vivo due to the movement of the blood. A flow-compensated measurement technique must be used. Another approach is to use a sample of extracted blood, but the $T_1$ value changes when exposed to the air, and as the erythrocytes begin to settle [187]. Oxygenation and mixing of the sample must be carefully controlled. This method cannot measure capillary blood $T_1$ which is different due to a difference in haematocrit between arterial, venous and capillary blood [187]. Published values for human brain vary from 1.4 s to 1.1 s for arterial blood with an average value ($n=6$) of 1.3 s [187, 170, 30, 230, 227, 72]. The recent work by Spees et al. [187] is perhaps the most reliable with careful control of oxygenation, pH, temperature, mixing and haematocrit. They measured arterial blood $T_1$ as 1.4 s and capillary blood $T_1$ as 1.7 s. This difference is due to changes in oxygenation and hematocrit. These changes could also occur during disease such as thrombosis. For improved accuracy capillary and arterial blood $T_1$ could be included separately in the model but this would increase the model complexity. For my estimates I use a fixed value of 1.4 s.

An alternative method of measuring $T_{1b}$ is to fit for it from ASL perfusion data. The impermeable FAIR solution, equation 3.25 has the fewest variables and will therefore probably be the most successful. The simulations (figure 3.8) predict that this solution is highly accurate in humans at 1.5T. Differentiating equation 3.25 with respect to measurement time, $t$, (equivalent to inversion time) we find that:

$$\frac{d\Delta M}{dt} = 2fm_o^0ae^{-\frac{t}{T_{1b}}}(1 - \frac{t}{T_{1b}})$$

Equating this to zero we find the maximum of the signal curve occurs at $t=T_{1b}$, as shown in figure 3.5 (with PS=0). Finding the time of the maximum perfusion signal therefore gives us an alternative method for measuring blood $T_1$. However, as shown in figure 3.5 the maximum is broad so this technique may not be very precise. This assumes that the arrival time for FAIR is zero. My attempts at implementing the FAIR technique produced very noisy images such that a maximum could not be reliably found.

### 4.5 Extravascular $T_1$

Extravascular $T_1$ can be approximated by a measurement of tissue $T_1$, i.e. the contribution of blood to tissue $T_1$ is small. This is justified by substituting typical grey matter parameters from Table 3.2 and relations from Table 3.1 into equation 3.29. I
find that $T_1$ is 1.6% greater than $T_{1e}$. Using the error propagation ratio of 0.23 from figure 3.11 this will produce a negligible error of 0.4% in perfusion.

Tissue $T_1$ was measured using a 3 point inversion recovery technique with gradient echo EPI collection in order to exactly match the image distortions of the perfusion images. Three image sets were collected with $TI = 1\, s$, $1.6\, s$ and one without inversion, $TR=7.2\, s$. With 8 averages for each set this takes a total of 3 min. The magnitude signal $S$ of the inversion images is given by:

$$S = S^0(1 - 2\beta e^{-\frac{TI}{T_1}})$$  \hspace{1cm} (4.3)

where $S^0$ is the magnitude signal from the image collected without inversion, $\beta$ is the degree of inversion ($\beta=1$ is perfect inversion) and $TI$ is the inversion time. Maps for $\beta$ and $T_1$ were calculated by fitting the equation to the data using least squares minimisation.

<table>
<thead>
<tr>
<th>Tissue mimicked</th>
<th>Calibration Values</th>
<th>Estimated $T_1$ values using inversion recovery technique</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$T_1$ at 19° $T_2$ at 19°</td>
<td>Scan1</td>
</tr>
<tr>
<td>-</td>
<td>1440</td>
<td>390</td>
</tr>
<tr>
<td>GM</td>
<td>959</td>
<td>223</td>
</tr>
<tr>
<td>WM</td>
<td>597</td>
<td>95</td>
</tr>
</tbody>
</table>

Table 4.1: $T_1$ measurements (column 4) were made on 3 chemical samples with known $T_1$'s (column 2). The measurement was repeated (column 5). The mean error in the $T_1$ measurement is 5.1%.

The technique was tested on a set of 3 chemical phantoms, gels made to a specific chemical formula to give them known $T_1$ values guaranteed to within 10%. Included were two phantoms with $T_1$ and $T_2$ values close to the values for grey matter (GM) and white matter (WM). The results are shown in Table 4.1. The mean error in $T_1$ measurement was 5.1% with a standard deviation of 3.8%, within the accuracy of the calibration of the phantoms. Using the error propagation ratio of 0.23 (figure 3.11) this will produce an error of 1.2% in perfusion.
TI measured by Griffin et al. & TI measured in this study

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>677</td>
<td>709</td>
</tr>
<tr>
<td>Median</td>
<td>650</td>
<td>728</td>
</tr>
<tr>
<td>Range</td>
<td>522 - 971</td>
<td>515 - 850</td>
</tr>
</tbody>
</table>

Table 4.2: $T_i$ measurements in 120 regions of white matter taken from 10 normal controls. Values in the second column taken from Griffin et al. [75].

A better test of the accuracy of the $T_1$ measurement is to compare the estimate to that found using a more accurate MRI technique. This can be done in vivo in the white matter, which has a very stable $T_1$ value. Ten normal volunteers were scanned using the $T_1$ technique as described above. For each volunteer 12 regions of interest (area 84 mm$^2$) were placed in the white matter. Small regions were placed in the middle of the white matter in order to avoid partial volume effects with grey matter or CSF. This protocol is very similar to that used by Griffin et al. [75] which used a $T_1$ technique measuring $T_1$ to an accuracy of 1.4% [155]. The volunteers had similar ages to those used in the study by Griffin et al. (mean age 32 years in this study compared to 36 years in Griffin et al.) and the same male to female ratio (7/3). The results are shown in table 4.2. The mean $T_1$ value measured by my technique is 4.7% greater than by the technique of Griffin et al. [75]. This could be partly due to differences in the anatomical location of the regions of interest.

4.5.1 $T_1_{sat}$

As discussed in section 3.2.6.3 the MT effects of the labelling pulse can reduce the extravascular $T_1$ to a value $T_1_{sat}$. Published values from another centre on an identical scanner using the CASL technique [4] estimate $T_1_{sat}$ as 746 ± 18 ms in grey matter and 525 ± 36 ms in white matter averaged across five subjects. These values are considerably shorter than the normal, unsaturated $T_1$ values of 1151 ± 12 ms for grey matter and 770 ± 11 ms for white matter, measured in the same subjects [4]. This error in $T_1_{sat}$, on the order of 50%, will propagate to give a 10% error in perfusion (using the ERP from figure 3.11). Alsop et al. [4] assume that when the labelling pulse is switched off the MT effects rapidly decay and $T_1$ returns to its unsaturated value. This is a complex issue requiring further work. However, it seems reasonable to assume that
with a delay time of around 1 s after the end of labelling, no MT effects remain and
the normal, unsaturated values of $T_1$ can be used in calculations. There will still be
an error however due to the presence of labelled molecules in the extravascular space
before the labelling is switched off. During this time the longitudinal magnetisation of
these molecules will relax with the relaxation time $T_{1sat}$.

### 4.6 Equilibrium arterial magnetisation

The equations for $\Delta M$ contain the parameter $m_0^a$, the equilibrium magnetisation of
arterial blood. It is possible to estimate this parameter from the unsubtracted control
image using the relation: $\lambda = M^0/m_0^a$ where $\lambda$ is the brain-blood partition coefficient
describing the fraction of water content in tissue compared to that in blood, and $M^0$ is
the equilibrium magnetisation of tissue.

The signal in the control image, $S_{\text{cont}}$, is given by:

$$S_{\text{cont}} = GM^0(1 - e^{-\frac{TR_{\text{ASL}}}{T_1}})e^{-TE/T2^*} \quad (4.4)$$

Where $T2^*$ is the transverse relaxation time of tissue, and $G$ is the arbitrary gain factor
of the scan. The $T_1$ map can be used with the $S_{\text{cont}}$ map (the control image) to produce
a map of $GM^0e^{-TE/T2^*}$. Since $TR_{\text{ASL}} \gg T_1$ ($TR_{\text{ASL}}=4s$) the error propagation from
$T_1$ to $S_{\text{cont}}$ is small, a ratio of only 0.06 for $T_1$ of 1 s.

The signal measured in the difference image, $S_{\text{diff}}$, contains the same gain factor
and signal loss due to $T2^*$ dephasing. But the $T2^*$ of the blood and extravascular
compartments could be different, so, for a full description of the signal we must refer
back to equation 3.13. The difference signal measured is given by:

$$S_{\text{diff}} = G(e^{-TE/T2^*}v_{eu}\Delta m_e(t) + e^{-TE/T2^*_b}v_{bw}\Delta m_b(t)) \quad (4.5)$$

where $T2^*_e$ is the $T2^*$ of extravascular space and $T2^*_b$ is the $T2^*$ of blood. Rearranging
equation 4.5 gives:

$$S_{\text{diff}} = Ge^{-TE/T2^*}(v_{eu}\Delta m_e(t) + \epsilon v_{bw}\Delta m_b(t)) \quad (4.6)$$

where

$$\epsilon = e^{-TE(T2^*_b/T2^*_e)} \quad (4.7)$$
Assuming $\epsilon \approx 1$ (which may not be the case, as discussed below) and that $T2_* \approx T2^*$ this gives:

$$S_{\text{diff}} = G e^{-TE/T2^*} \Delta M$$  \hspace{1cm} (4.8)

and, using the definition of $\lambda$:

$$\frac{\Delta M}{m_0^a} = \frac{\lambda S_{\text{diff}}}{G M_0^0 e^{-TE/T2^*}}$$  \hspace{1cm} (4.9)

As described above, the control image gives us a map of $GM_0^0 e^{-TE/T2^*}$, which can be used with a value of $\lambda$ to convert the difference signal $S_{\text{diff}}$ into a map of $\Delta M/m_0^a$. The $\Delta M/m_0^a$ map can be used to calculate a perfusion map using one of the model equations from Chapter 3.

One issue here is whether to use a map of $GM_0^0 e^{-TE/T2^*}$, rather than an average whole brain value. As Buxton et al. point out [29] the use of a map also requires the use of a $\lambda$ map which is known to vary by 15% across the brain [169]. Alternatively, it can be assumed that $G$ and $T2^*$ do not vary much across the brain and a whole brain average of $GM_0^0 e^{-TE/T2^*}$ can be used with a whole brain average of $\lambda$. I chose this latter option with $\lambda=0.9$ [169]. Note, that this gives $\lambda$ a high error propagation ratio of about 1.

The assumption that $\epsilon=1$ is only true if TE is short compared to $T2^*$ or if $T2_* = T2^*$. In my implementation of CASL, TE=34ms which is not small compared to $T2^*$, so the assumption is inaccurate if $T2_* \neq T2^*$. A recent experiment by Spees et al. [187] (discussed in section 4.4) measured $T2_*$ to be approximately 40ms at TE=34ms. Tissue $T2^*$ (assumed to be equivalent to $T2_*$) were measured by Bandettini et al. [11]. They measured $T2^*$ in 4 subjects, as 61ms, 37ms, 44ms and 51ms (showing the large individual variation in the values), giving a mean value of 48ms. This is fairly similar to $T2_*$ but will nevertheless cause an error to the perfusion estimate. Using $T2_* = 40$ms, $T2^* = 48$ms and $TE = 34$ms in equation 4.7 $\epsilon = 0.87$. Considering this value in equation 4.6 shows that the signal is effectively weighted towards the water in the extravascular compartment.

If $\epsilon \neq 1$, the error in $\Delta M$, (and therefore perfusion) is given by

$$\text{error} = \frac{(v_{w} \Delta m_{e}(t) + \epsilon v_{bw} \Delta m_{b}(t)) - \Delta M}{\Delta M}$$  \hspace{1cm} (4.10)
The error will depend on the relative proportion of labelled molecules in the blood \((v_{bw}\Delta m_b(t))\) and extravascular \((v_{ew}\Delta m_e(t))\) space, which will depend on the measurement time. Using the \emph{slow} solution of the two-compartment model (equations 3.22, 3.23 and 3.24) the error was calculated for \(\epsilon=0.87\) using the fixed values: perfusion = 60 ml blood min\(^{-1}\) (100 ml tissue)\(^{-1}\), \(PS/v_{bw}=30\) min\(^{-1}\), \(t_A=0.5\) s, \(T_{1b}=1.4\) s, \(T_{1e}=1\) s, and \(t_e=1.7\) s, chosen to match the experimental conditions. \(\Delta M\), and therefore perfusion is underestimated by 9% at zero delay time, 6.6% at 1s delay and 5% at 1.5s delay.

To correct for these errors a separate measurement of \(T2^*\) is required. The inter-subject variability in \(T2^*\) shown by Bandettini et al. [11] suggests that a fixed estimate would be inaccurate. Alternatively, the error can be reduced by reducing the echo time of the sequence. If spin echo EPI is used the error is also significant. \(T2\) values are longer but quite different between blood and tissue (typically 80ms in tissue, 200ms in blood [79]). In this case the signal is weighted towards water in the blood compartment.

This error also affects the shape of signal curve, which has implications for curve fitting. This is discussed in section 4.9. Further work could look at the inclusion of a \(T2^*\) tissue measurement.

### 4.7 Arrival time

The model must account for longitudinal relaxation of the blood signal before it enters the tissue voxel. This requires a measure of arrival time \(t_A\), the time for blood to travel between the labelling plane and the tissue voxel. There is some debate in the literature as to whether this is the time to reach the tissue voxel or the time to reach the capillary bed [4]. This is due to confusion over the location of the labelled protons once they have entered the voxel; a single compartment model would wrongly assume they mix instantaneously across the whole tissue. To correct for this a second arrival time to the capillary bed would signify the start of mixing. By including both arrival times [4] the model is equivalent to a two-compartment model with an exchange time \(T_{ex}\) as explained in section 3.2. Further work [73] suggests a distribution of arrival times should be used due to the spread of blood velocities and different blood trajectories.

In my two-compartment model we require only the time to reach the tissue voxel.
Any subsequent movement of labelled protons across the capillary wall is dealt with by the permeability term included in the model. One limitation of the two-compartment model is that an average PS value is assigned to the voxel. In reality there will be a range of PS values corresponding to a range of vessel sizes. This is a consequence of assuming a well-mixed blood compartment.

Methods
The CASL signal curve is most sensitive to arrival time during the wash-in of the labelled blood, i.e. for measurement time \( t \leq t_A + t_L \), parts 1 and 2 of figure 2.7. The transition between part 1 and 2 is the most sensitive time, when the labelled blood first enters the voxel. However, we are constrained to measurement times greater than the labelling time \( t_L \) since measurements cannot be made during labelling. A short labelling time of 192 ms (one labelling block) was chosen in order to allow the minimum measurement time and the maximum chance of measurement before the labelled blood enters the voxel. Measurements were taken in two subjects (male age 27, female age 25) at a range of nine delay times from 0 to 1 s. This gives measurement times of 193 - 1200 ms plus imaging time which varies from 38 to 577 msec depending on slice number. Measurements were made in seven slices with 5 mm slice thickness and 10 mm gap with the labelling plane positioned 2 cm below the lowest slice. Three image sets for a T1_e map were also collected as described in section 4.5 on extravascular T_1.

Analysis
A short measurement time also has the advantage of improved accuracy of the impermeable solution. The accuracies of the model will be close to those calculated for FAIR at measurement time of 1 s as shown in figure 3.8. However, for estimates of arrival time the result will be less dependent on the type of model since it is more a question of detecting when the signal begins to increase. The average difference signal across each slice at each delay time was recorded. The impermeable model was fitted to the data using least squares minimisation, giving values for perfusion and arrival time. Fixed values were: \( T_{1e}=1.4 \) s, \( \alpha=0.7 \) and \( Gm_0 \) estimated as described above. To calculate random errors a Monte Carlo bootstrapping technique was used to resample the data based on measured errors in the data. These errors were found by performing scan-rescan tests, as described in Chapter 5, giving raw signal reproducibility (95% confidence limit) of about 8% for a single slice at a delay time of 0.75s. One hundred
sets of resampled data (resampling the data according to the known errors given above) from each data-set were used to find the standard deviation in the fitted parameters for each original data-set. Examples of the fitting are shown in figure 4.3.

![Graph showing fitting results](image)

**Figure 4.3:** Fitting the two-compartment solution (equations 3.22, 3.23 and 3.24) for perfusion and arrival time. The data in blue show the average difference signal for a tissue slice located 8 cm from the labelling plane. In red are the data for a slice 11 cm from the labelling plane. The error bars show the 95% confidence limit in the difference signal from scan-rescan tests.

**Results**

Figure 4.4 shows the results of the arrival time fitting. Arrival time increases linearly with distance from the labelling plane suggesting that the arterial blood travels with a constant velocity to all the slices. Least squares fitting of a straight line constrained at the origin gives the velocity as 12.0 cm s$^{-1}$ for subject 1 and 13.0 cm s$^{-1}$ for subject 2, giving a mean value of 12.5 cm s$^{-1}$. This velocity is typical for small arteries. Results are consistent between the two subjects with 8% difference in estimated blood velocity. Results are also in reasonable agreement with published values for arrival time [4, 73, 225]. One study using FAIR showed a similar linear relationship between arrival time and distance but gave a lower velocity of approximately 10 cm s$^{-1}$ [225]. In both subjects there is an anomalous result at slice distance of 3.5 cm. The data for this slice are collected towards the end of EPI acquisition giving a shortest measurement time of 546 ms. It is likely that this is greater than the true arrival time, making the estimate inaccurate since the labelled blood arrives before the start of signal measurement. This
Arrival time estimates with distance from labelling to image slice. For both subjects we find arrival time increases linearly with distance, corresponding to velocities of 12 cms$^{-1}$ (subject 1) and 13 cms$^{-1}$ (subject 2). The anomalous point at 3.5 cm is explained in the text. The error bars represent 2 x the standard deviation in the 100 arrival time estimates from the re-sampled data. Straight lines, constrained at the origin, are fitted to the data.

could be tested by changing the slice collection order.

As well as changing with slice distance, arrival time will also change within the slice. The signal to noise ratio of our data was insufficient to accurately estimate arrival time on a pixel by pixel basis. For large regions of grey and white matter I could detect no significant difference in arrival time.

### 4.8 Perfusion

We can now use all of the measured or assumed parameters described in the previous sections to estimate perfusion. The three models, the single compartment, impermeable and two-compartment solution as described in the Methods section 4.1 are compared. For the two-compartment solution I use a value for PS/$v_{bw}$ of 1.5/0.05 = 30 min$^{-1}$. For the single compartment model I assume $T_{1,app}=T_1$ since, as described in section 3.3 this makes negligible difference to the perfusion estimate. In practice the two-compartment solution can be used for all of the modelling with PS/$v_{bw}$ set to zero (impermeable model), 30 min$^{-1}$, and $\infty$ (single compartment model).
Methods
A single subject (female, age 26) was scanned at a range of 6 delay times: 0, 0.1, 0.2, 0.4, 0.8 and 1.6 s with labelling time 1.73 s. Scan parameters were as described in the Methods section 4.1 with resolution 32x32, FOV 16cm, slice thickness 5mm and gap 3mm. These scan parameters were chosen to give isotropic voxels of length 5mm such that the many-vessel theory of section 3.4 can be compared to the results. Three image sets for a T1ε map and Gm0 were also collected as described in section 4.5 on extravascular T1. Fixed parameters used in the perfusion calculation were: α=0.7, T1b=1.4s and tA=x/12.5 s where x is the distance from the labelling plane to the slice in cm. Perfusion, the only remaining unknown, was calculated on a pixel by pixel basis at each delay time for each model.

Segmentation
The perfusion maps were segmented into regions of grey matter and white matter using

Figure 4.5: Segmentation. Top left shows a quantitative T1 map, and top right a quantitative perfusion map. The bottom images show the automatic segmentation into white matter (left) and grey matter (right) based on T1 values.

the T1 map. The T1 boundaries were: 500 - 900 ms for white matter, 1000 - 1400 ms for grey matter, chosen on the basis of published T1 values [4, 188, 206] and such that the segmentation looked reasonable by eye. Typical segmented regions are shown in figure 4.5 along with calculated T1 and perfusion maps (at 0.8 s). Voxels with negative
values on the subtraction image (control - label) were excluded, causing a few gaps within the white matter as seen in figure 4.5. The grey matter segmentation tended to include a few voxels outside the brain. The technique of eroding the outside layer of the segmented area and then dilating out to the edge of the brain [60] could help to remove some of these isolated voxels. Note that since perfusion is an intensive quantity it is not essential to include all tissue in the measurement; it is more important that the tissue is pure. The mean perfusion estimate for each tissue type over all slices at each delay time was calculated.

Results
The results of the perfusion estimates for each model at each delay time are shown in figure 4.6. Of course, true capillary perfusion will be the same regardless of delay time, so any dependence on delay time shows an error in the perfusion estimate. Two effects can be seen: An initial drop in the perfusion estimate at short delay times and a rise in the white matter perfusion estimate at longer delay times.

Initial drop
The perfusion estimate decreases with delay time up to 0.5 s, irrespective of the model choice. This is in accordance with the many-vessel simulations of section 3.4, indicating the through-flow of blood in large vessels. The critical delay time appears to be shorter than predicted - about 500 ms rather than 800 ms. The perfusion estimate drops immediately from zero delay time in all voxel types, without the flat section predicted by the many-vessel model shown in figure 3.16. These two observations can be explained if we consider the position of the labelled blood at zero delay time. In the model I assumed that labelled blood had to cross the whole distance of the voxel before it could exit, producing the flat section of figure 3.16. In practice, at zero delay time the voxel is full of labelled blood which will immediately begin to exit with increasing delay time. On average, labelled blood will only have to cover half the distance of the voxel to exit and so the critical delay time should be half that predicted, i.e. 400 ms. The results show good agreement with this.

White matter increase
Figure 4.6 shows that the white matter perfusion estimates are most affected by the choice of model. At a delay time of 0.8 s there is a 63% increase in perfusion estimate
Figure 4.6: Perfusion estimates using three different models, a) The single compartment model, equations 3.6, 3.7 and 3.8; b) The simplified Slow model, equations 3.22, 3.23 and 3.24; and c) the Impermeable model, equations 3.26, 3.27 and 3.28. The models are used to calculate perfusion at each delay time, shown by the data points. The lines simply join the data points to aid vision.
with the single compartment model when compared to the two-compartment solution, as predicted in the simulations of section 3.2.5.1. For the single compartment model the white matter perfusion estimate rises with increasing delay time which I believe must be an artefact of incorrect modelling. The single compartment model assumes the perfusion signal decays with the $T_1$ of tissue, which for white matter is likely to be too low since tissue $T_1$ is much lower than blood $T_1$. So the predicted decay of signal with delay time is too fast. This would cause an artefactual overestimation of perfusion with increasing delay time, as we see here. The grey matter perfusion estimate is less affected by model choice but is slightly lower with the two-compartment and impermeable models. At a delay time of 0.8 s there is a 10\% increase in perfusion with the single compartment model when compared to the two-compartment solution, slightly lower than predicted in the simulations of section 3.2.5.1. This is due to the larger grey matter $T_1$ values included in the segmentation when compared to the value of 1.0 s used in the simulation.

Figure 4.7: Perfusion maps at delay time=1.6 s using the single compartment model (left), the slow two-compartment model (middle) and the impermeable model (right).

The models produce quite different perfusion maps with a delay time of 1.6 s as seen in figure 4.7. With the single compartment model we lose grey/white matter contrast.

The two-compartment model gives perfusion estimates of 33, 83 and 73 ml blood min$^{-1}$ 100 ml tissue$^{-1}$ for white matter, grey matter and whole brain respectively, at a delay time of 0.8 s. Published perfusion values vary widely and there is large variation between subjects, as discussed in Chapter 5. These CASL values fall within the range of typical published values using techniques of PET and Gadolinium bolus tracking [116, 31].
4.9 The permeability parameter, $PS/v_{bw}$

The results of the previous section on perfusion suggest that the difference signal data at a range of delay times could be used to fit the two-compartment solution for $PS/v_{bw}$.

Methods

Images were collected from three subjects (female, mean age 28 yrs) and repeated on one subject in order to test reproducibility. Scan parameters were as described in the Methods section 4.1 with 8 slices of 7mm thickness and 2mm gap and six delay times from 0.25 to 1.5 s. By fitting first all six points and then only the later 5 points it should be possible to assess the effect of large vessel through-flow. As shown in the previous section, by 0.5 s there should be no large vessel signal remaining. Fixed parameter values were: $\alpha=0.7$ and $T_{1e}=1.4s$. $T_{1e}$ was set at either a saturated or normal value in order to assess the effect of MT, as described below. The difference images were segmented into grey and white matter, as described in the previous section. Data from single pixels in the vicinity of large vessels were also recorded. They were identified as having high signal in both the $T_1$ and perfusion difference images.

Analysis

The single compartment solution, equations 3.6, 3.7 and 3.8, and the two-compartment solutions, equations 3.22, 3.23 and 3.24, were fitted (using least squares minimisation) to region of interest data (grey or white matter, or single pixels near large vessels) from each slice for the free parameters $f$, $PS/v_{bw}$ and $t_A$. Four sets of fitting were carried out:

1. with $T_{1e}$ set to $T_{1, sat}$ (0.75 s grey matter, 0.52 s white matter [4]) and 6 data points (0.25 - 1.5 s).

2. with $T_{1e}$ set to $T_{1, sat}$ and 5 data points (0.5 - 1.5s).

3. with $T_{1e}$ set to normal values $T_{1, norm}$ (1.1 s grey matter, 0.8 s white matter [4]) and 6 data points (0.25 - 1.5 s).

4. with $T_{1e}$ set to $T_{1, norm}$ and 5 data points (0.5 - 1.5s).

The uncertainties on the fixed parameters were estimated by considering a range of literature values [170, 4, 3] and were estimated as: $T_{1b} \pm 0.1 s$, $T_{1e} \pm 0.1 s$, $\alpha \pm 0.1$. These uncertainties were propagated to produce systematic errors in the fitted parameters for
analysis set 1 only. For the grey and white matter regions the *slow* two-compartment solution was used and for the large vessel pixels the *fast* two-compartment solution was used with the extra parameter \( v_b = 0.05 \) (equations 3.23 and 3.24).

To calculate random errors a Monte Carlo bootstrapping technique was used to resample the data based on measured errors in the data. These errors were found by performing scan-rescan tests as described in Chapter 5. For similar volumes as used here, grey matter reproducibility (95% confidence limit) is 8.6% (volume 42 cm\(^3\)) and white matter 14% (volume 8.4 cm\(^3\)) at a delay time of 0.75s as shown in table 5.2. One hundred sets of resampled data (resampling the data according to the known errors given above) from each data-set were used to find the standard deviation in the fitted parameters for each original data-set. Average values for perfusion and \( PS/v_{bw} \) across all slices were found by calculating a weighted mean of values from each data-set. Weights were set as the inverse of the variance for each measurement.

**Results**

Figure 4.8 shows the typical performance of the single and two-compartment models when fitting for perfusion, arrival time and \( FS/v_{bw} \). They show that the two-compartment model fits well to both extremes of data, i.e. white matter and tissue containing large vessels. It can be seen that the single compartment model decays too quickly to fit the white matter data decay (Figure 4.8b) presumably because the labelled water remains in the blood compartment with slower longitudinal relaxation. In some cases the single compartment model compensates for the inability to fit by shifting the curve and overestimating the arrival time, as shown in Figure 4.8c. For the single pixels containing large vessels (Figure 4.8d) the single compartment model decays more slowly than the data, presumably because of outflow of non-exchanging molecules from the blood compartment.

**Perfusion results**

Table 4.3 shows the results of the fitting for perfusion averaged over all data sets (as described in Methods section) for three subjects. There is a large variability in perfusion values between subjects from 60 to 93 ml blood min\(^{-1}\) (100 ml tissue)\(^{-1}\) in grey matter and 22 to 30 ml blood min\(^{-1}\) (100 ml tissue)\(^{-1}\) in white matter. Random errors are low, typically about 3% but systematic errors are higher, around 16%. The values
Figure 4.8: Three parameter fits for perfusion, arrival time and PS/v bw using the two-compartment solution (two), equations 3.22, 3.23 and 3.24 and the single compartment solution (single), equations 3.6, 3.7 and 3.8. All results are from subject 1, a) region of grey matter slice 3, b) region of white matter slice 4, c) region of white matter slice 3, d) single pixel containing large vessels, slice 3. The large vessel data, d) was fitted using the fast two-compartment solution, the rest with the slow two-compartment solution. The two-compartment model produces a better fit than the single compartment model for all extremes of data and always lies within the data error bars. The error bars on the x axis span 80 ms, the time for data collection. The error bars on the y axis show the 95% confidence limit on signal measurement taken from scan re-scan reproducibility tests.

when fitting only the later 5 points compared to the full 6 points (analysis set 1 with 2 and 3 with 4) are not significantly different. This suggests that the effect of large vessel through-flow is small for delay times greater than 0.25 s. Changes to T1c however did
Table 4.3: Estimated perfusion values (ml blood min$^{-1}$ (100 ml tissue)$^{-1}$) in three subjects. The four data sets correspond to the 4 types of analysis as described in the analysis section. The random error is shown in brackets as two times the standard deviation due to noise in the data as calculated using a monte carlo method. For analysis 1 the systematic errors are shown in square brackets. These are errors due to the uncertainties in the fixed parameters. Perfusion values are found to be relatively independent of the delay time range and the value of $T_1$. 

sometimes produce significantly different perfusion values in the grey matter of subject 1 (comparing analysis 1 with 3). Looking at table 4.4 it can be seen that this only happens when there is also a large change in the fitted $PS/v_{bw}$ value. With a high $PS/v_{bw}$ value perfusion increases by about 10%. The repeat scan on subject 1 shows high perfusion reproducibility of about 5%, which is within the random errors of the fitting.

**PS/v$_{bw}$ results**

Table 4.4 shows the corresponding $PS/v_{bw}$ results. There is a large variation in $PS/v_{bw}$ between subjects, tissue types and analysis techniques. Both random and systematic errors are large, on the order of 100%, and reproducibility is poor, indicating that this is not a very reliable method for measuring $PS/v_{bw}$. White matter values (mean value over 3 subjects = 3 min$^{-1}$) are consistently lower than grey matter values (mean value = 15 min$^{-1}$, excluding the three extreme values). This was initially unexpected since the parameter reduces to $2P/rv_{bw}^b$ with all components being tissue independent. However, this contradiction could be explained if we consider the location of the blood in the
Table 4.4: Estimated $PS/\nu_{bw}$ values ($\text{min}^{-1}$) in three subjects. The four data sets correspond to the 4 types of analysis as described in the analysis section. The random error is shown in brackets as two times the standard deviation due to noise in the data as calculated using a monte carlo method. For analysis 1 the systematic errors are shown in square brackets. These are errors due to the uncertainties in the fixed parameters. $PS/\nu_{bw}$ values are quite sensitive to delay time range and $T1_e$ values, especially in grey matter. Errors on $PS/\nu_{bw}$ are large.

different tissue types when the measurement is taken. For white matter it is thought [225, 4] that the blood takes longer to reach the capillary bed than in grey matter so we could be measuring $PS/\nu_{bw}$ of the arterioles rather than the capillaries, which, as found, would be lower. This is supported by the trend towards higher $PS/\nu_{bw}$ values when only the later 5 data points are fitted since at later times more of the labelled molecules will have moved down the vasculature to increasingly permeable vessels. The measurement indicates the average value of $PS/\nu_{bw}$ over all vessels in the voxel.

It would be interesting future work to apply mild diffusion weighting gradients to the pulse sequence [230]. This will remove some signal from blood in larger vessels. The theory would predict that the PS value would increase with application of these gradients due to a higher proportion of the signal originating from capillary blood.

The values for $PS/\nu_{bw}$ estimated from the later 5 time points are likely to be more accurate estimates of true capillary permeability, since the labelled molecules have had more time to move into the capillaries. The mean grey matter value over 3 subjects for 5 time points, excluding the three extreme values, is $20 \text{ min}^{-1}$. This agrees reasonably
well with published values but is a little on the low side. For example Herscovitch [86] found PS of 1.5 min$^{-1}$ (grey matter) giving PS$/v_{bw}$ values of approximately 30 min$^{-1}$ using typical blood volume of 5% [116]. It is worth noting here that the exact value of PS$/v_{bw}$ is unimportant due to the very low error propagation ratio (figure 3.11). What is important is establishing whether PS$/v_{bw}$ is finite (rather than infinite as in the single compartment model) which will have a large bearing on the perfusion estimate.

**T2* effects**

Another possible cause of PS$/v_{bw}$ underestimation is the error due to neglecting the compartmental effects on T2*. As discussed in section 4.6, this will cause an underestimation of ΔM which decreases with increasing delay time. i.e. the measured signal decay curve is shallower than predicted by ΔM. Since a reduction in PS$/v_{bw}$ also produces a shallower curve (more spins relaxing at the longer T$_i$ of blood) this could cause an underestimation of PS$/v_{bw}$.

**MT effects**

Comparing analysis sets 1 and 2 with 3 and 4 for the grey matter data of subject 1 shows that the value of T$_{1e}$ dramatically changes the estimated PS$/v_{bw}$ value. With T$_{1e}$ = 1100 ms the data were better fit by the single compartment model on three occasions, but with large random errors. In this situation there is little difference between the two model curves since the extravascular and blood T$_1$ values are so close (1.1 s and 1.4 s). Remember that if T$_{1e}$ = T$_{1b}$ the models are indistinguishable. Hence the fitting is unreliable, with large random errors. On these occasions there was a parallel increase in perfusion showing the effect the model choice has on the perfusion estimate.

It is important to note that the model can fit to all types of blood flow from true capillary perfusion to blood flow in a single large vessel, and will simply measure ‘perfusion’ as the total volume of blood flowing through a tissue volume per unit time. The insensitivity of perfusion estimates to increasing delay time after 250 ms (shown in the comparison of fitting 5 and 6 data points) suggests that after this time all of the blood present in the voxel will go on to perfuse that voxel. It is important to remember that any blood which will eventually perfuse the measurement voxel should be included, even if it is in larger arterioles at the time of image collection.
Figure 4.9: Arrival time results from the three parameter fits for perfusion, arrival time and PS/vbw. Average results from all 3 subjects and the repeat measurement are shown. Arrival time is found to be approximately linear with distance from labelling plane (regression lines shown) with white matter arrival time being greater than grey matter arrival time. The error bars show the standard deviation between measurements.

Arrival time results

The decaying part of the signal curve is very insensitive to arrival time producing large random errors in this measurement. In fact with PS/vbw = 0 (the impermeable solution) the decay curve is independent of arrival time. However, average results from all three subjects using analysis set 3 (figure 4.9) shows the same linear result as in section 4.7. The slope of the curve gives an average velocity of about 10 cm/s, lower than the results in section 4.7. This could be due to greater dispersion of the labelled bolus at these later measurement times. The white matter arrival time is found to be longer than the grey matter arrival time which could be due to lower blood velocity in the feeding vessels [225, 4]. The measurements from section 4.7 provide a more reliable measurement of arrival time.

This section shows that the two-compartment model provides a better fit to the ASL data than the single compartment model. Despite this, studies comparing ASL perfusion estimates using a single compartment model to those using other techniques do not show the expected errors in perfusion. One study in cats at 4.7T made comparisons with microspheres [237], finding ASL underestimated perfusion with increasing deviation at higher flow rates, in agreement with my findings (figure 3.7). A study
in humans at 1.5T [228] made comparisons with PET and found good agreement in grey matter but an underestimation of white matter perfusion with ASL, contrary to my model predictions (figure 3.7). As the authors suggest, this may have been due to an underestimation of white matter arrival time. A third study [119] comparing ASL to bolus tracking found reasonable agreement but large inter-subject variation in this agreement for different regions of interest. These results do not all show the over- and underestimations that my model predicts, but they are also all fairly inconsistent. This highlights the problem of comparing different perfusion techniques, in that none of them can be assumed to be the 'gold standard'. With each technique there are various factors which could cause an error to perfusion quantification. An alternative approach is to validate perfusion models by fitting to data from a single technique, e.g. using varying delay time as shown here.

4.10 Summary

- Published values give inversion efficiency, $\alpha = 0.7$ and the $T_1$ of blood, $T_{1_b}=1.4$ s.

- A three point inversion recovery technique measures $T_{1_e}$ with 5% accuracy. This can be used in conjunction with the perfusion control images to measure $G\alpha_0$.

- By varying the delay time it is possible to measure the difference signal at several time points in order to fit for the permeability parameter $PS/v\omega$ and the arrival time $t_A$ as well as perfusion.

- Arrival time is found to be linear with distance from the labelling plane. This suggests that blood travels in the feeding vessels with a constant mean velocity, measured as 12.5 cms$^{-1}$.

- The signal from large vessel through-flow is negligible for delay times greater than 500 ms. A look at recent applications of CASL [212, 57, 70, 106, 4] shows the use of delay times between 1 and 1.5 s. My work shows that a lower value could be sufficient and will improve the signal to noise ratio of the data. Longer delay times will however also reduce arrival time sensitivity.

- The two-compartment model provides a better fit to data than the single compartment model. The largest difference is seen in white matter. Grey matter data is less sensitive to model-type since $T_{1_e}$ is closer to $T_{1_b}$.

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Figure 4.10: Quantitative perfusion images from a single measurement at delay time 0.75s. Imaging parameters: labelling time 1.7s, 45 averages. Quantification parameters: \( \alpha = 0.7 \), \( T1_b = 1.4 \), \( t_A = \text{slice distance (cm)}/12.5 \), \( PS/v_{bu} = 30 \text{ min}^{-1} \).

For clinical scanning it is not practical to measure the perfusion signal at several time points due to limited scanner time. A single timepoint measurement along with the \( T_1 \) scans gives a scan time of as little as 9 minutes; highly desirable. In order to quantify perfusion from a single time point, values for the other 6 parameters are required. \( \alpha \) is assumed to be 0.7; \( T1_b \) is assumed to be 1.4 s; \( Gm_0 \) and \( T1_e \) can be measured; \( t_A \) is assumed to be linear with distance from the labelling plane with a velocity of 12.5 cm s\(^{-1}\); \( PS/v_{bu} \) can be set to 30 min\(^{-1}\) (using \( PS = 1.5 \text{ min}^{-1} \) and \( v_{bu} = 0.05 \)). As shown using error propagation in figure 3.11 the exact value of \( PS/v_{bu} \) is not important.

Using these parameters, and choosing a delay time greater than 0.5 s, a single measurement should give an accurate perfusion estimate. Figure 4.10 shows typical quantitative perfusion images using a delay time of 0.75s. In the next 2 Chapters I show results of using this protocol on both patients and normal volunteers.
Chapter 5

Perfusion measurements in normal subjects

In this chapter the nature of perfusion variations is considered on all scales. First, the reproducibility of the technique is measured in order to put confidence limits on later measurements. Perfusion is measured in 32 normal subjects in order to assess the inter-subject variability and age and gender dependence. These measurements are important for later perfusion comparisons between normal subjects and patients. Large perfusion variation between normals will indicate the number of subjects required in order to measure a significant perfusion change. Any age and gender effects are of physiological interest and should be controlled for in clinical studies.

The variation of perfusion with time over a day and a week in a single subject is presented - to my knowledge the first study of its kind due to the invasive nature of other measurement techniques. It might be expected that these variations will be large since there is large capacity for perfusion change in the brain. Studies of perfusion reserve capacity under acetazolamide challenge show perfusion increases of about 50% [77, 41], similar to the regional perfusion increase during task activation [227, 230, 110]. If individual perfusion variations are large it may be necessary to take several perfusion measurements in each subject for a reliable mean perfusion measurement.

Regional correlations in perfusion within the brain are investigated. Temporal correlations in perfusion between both neighbouring and more distant voxels could indicate functional connectivity [36, 19, 122]. Published measurements in the heart and the lung show that local spatial correlations in perfusion follow a fractal model. This model was
tested in the brain.

5.1 Reproducibility within a subject

Imperfect reproducibility comes from three sources - instrumental errors, repositioning errors, and biological variation. Instrumental errors include any non-perfusion change between the two scans that could cause a change to the perfusion measurement, e.g. labelling efficiency, arrival time, gradient homogeneity. Since the 8 slices do not cover the whole brain, any slight shift in positioning will change the tissue included in the measurement, producing repositioning errors. Real biological variation in perfusion will also be included in a standard scan-rescan test of reproducibility. This standard technique will measure all three contributions together. It is possible to remove the repositioning error by using continual scanning while the subject remains in the scanner. Biological variation can be removed by careful separation of the perfusion averages that make up the perfusion measurement. For example, imagine there are no instrumental

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure5.1.png}
\caption{Possible perfusion variation over 12 minutes.}
\end{figure}

or repositioning errors and that figure 5.1 shows the real perfusion variation over 12 minutes (the time for two perfusion scans). If the 90 averages are split in half down the middle the second scan will show increased perfusion. If the averages are separated in an interleaved fashion one scan may also show increased perfusion due to regular fluctuations in perfusion (as reported in several studies [105, 122]). Random separation of the averages should remove biological variation.
5.1.1 Methods

Scanning
Ten subjects (7 female, 3 male, mean age 27.9 sd 5.4 years) were scanned using the
scan parameters as described in section 4.1. Labelling time was 1.73s, delay time 0.75s,
slice thickness 6mm with 3mm gap, 90 averages. 3 image sets were collected for a $T_1$
measurement as described in section 4.5. This gave a total scan time of 15 minutes.
The subject was removed from the scanner and then repositioned. Care was taken to
reposition the subject at the same position in the scanner and to prescribe the slices
in the same location. The scanning was repeated but with only 45 averages for the
perfusion scan, giving a further scan time of 9 minutes.

Analysis
The $T_1$ map was calculated as described in Chapter 4 section 4.5. The $T_1$ map was
registered to the control (i.e. unsubtracted) perfusion images [192] in order to remove
any motion artefacts between the two image sets. Rigid registration was used to correct
for in-plane shifts and rotations. The registration works by minimising the difference
in signal intensities between the two images in an iterative way over the whole image.
Quantitative perfusion maps were calculated using the simplified slow two-compartment
model; equations 3.22, 3.23 and 3.24 from Chapter 3. Fixed model parameters were:
$\alpha=0.7; \, T_{1w}=1.4s; \, arrival \, time \, t_A$ is assumed to be linear with distance from the la­
belling plane with a velocity of 12.5 cms$^{-1}$; $PS/v_{bw}=30 \, \text{min}^{-1}$. The perfusion maps
were segmented on the basis of $T_1$ values: 0.5 - 0.8s for white matter, and 1.0 - 1.4s for
grey matter. This fairly narrow range of $T_1$ values was chosen in order to include only
relatively pure grey and white matter.

Scan re-scan - to include instrumental, repositioning and biological changes.
The perfusion values from the final 45 averages of the first scan were compared to the
perfusion values from the second scan. The differences in perfusion will be due to in­
strumental errors, repositioning errors and real perfusion changes between the two scans.

Continual Scanning - to include only instrumental errors.
The repositioning error can be removed by splitting the first scan of 90 averages into
two halves and comparing the perfusion measurement from each half. By randomly
separating the 90 averages into two halves biological errors will be minimised.
5.1.2 Results

5.1.2.1 Scan re-scan

Figure 5.2: Perfusion reproducibility results from ten subjects. Percentage perfusion change between first scan and second scan after repositioning. Imperfect reproducibility is a result of instrumental and repositioning errors, and real biological variation.

Table 5.1: Perfusion reproducibility results from ten subjects. The standard deviation (sd) of the signed differences from figure 5.2 can be converted into the standard deviation of the underlying population. From this the 95% confidence limit on the perfusion measurements can be calculated.
Figure 5.2 shows the results of the scan re-scan test for the ten volunteers over the three tissue types, white matter, grey matter and whole brain. The reproducibility of the ratio of grey to white matter perfusion is also calculated. Table 5.1 gives the standard deviations of the differences while retaining the sign. The fractional uncertainty in the standard deviation of N values is given by $1/\sqrt{(2(N-1))}$ [194], which for our case of 10 values is 0.24. Following the methods of Bland and Altman [20] the standard deviation of the difference between repeats (sd) can be used to find the standard deviation of the underlying population (s): $s=sd/\sqrt{2}$. The 95% confidence limit is then $1.95$ times this value. These values are also shown in table 5.1 and will be used in later analyses. The uncertainty of the original sd calculation propagates linearly to the 95% confidence limit.

5.1.2.2 Continual scanning

<table>
<thead>
<tr>
<th>Vol. (cm$^3$)</th>
<th>White Matter</th>
<th>Grey Matter</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% error</td>
<td>Absolute error ml/min/100 ml</td>
</tr>
<tr>
<td>169</td>
<td>-</td>
<td>$5.8\pm1.4%$</td>
</tr>
<tr>
<td>42</td>
<td>$7.4%\pm1.7%$</td>
<td>$1.8\pm0.4$</td>
</tr>
<tr>
<td>8.4</td>
<td>$14%\pm2.1%$</td>
<td>$3.5\pm0.5$</td>
</tr>
<tr>
<td>4.2</td>
<td>$21%\pm2.6%$</td>
<td>$6.5\pm0.8$</td>
</tr>
<tr>
<td>1.4</td>
<td>$43%\pm4.9%$</td>
<td>$8.3\pm1.0$</td>
</tr>
<tr>
<td>0.34</td>
<td>$77%\pm7.5%$</td>
<td>$11.5\pm1.1$</td>
</tr>
<tr>
<td>0.084</td>
<td>$110%\pm9.4%$</td>
<td>$18.5\pm1.6$</td>
</tr>
</tbody>
</table>

Table 5.2: Perfusion reproducibility for different volumes of white and grey matter. The table shows the 95% confidence limits of the perfusion measurements. Results are expressed as a percentage and an absolute error in the perfusion measurement. Imperfect reproducibility is due to instrumental errors only.

Table 5.2 shows the results of the continual scanning with random separation of the 90 averages. That is without the errors due to repositioning and perfusion drift. The 95% confidence limits were calculated using the same statistical method as in the previous section, table 5.1. The reproducibility of a range of different tissue volumes were found.
A volume of 169 cm$^3$ corresponds to whole brain grey matter and 42 cm$^3$ to whole brain white matter, using the T$_1$ segmentation technique as described in the methods section. Volume 0.084 cm$^3$ corresponds to a single voxel. The 95% confidence limits for whole brain white matter and grey matter are not significantly different from the scan-rescan test described in the previous section. This shows that the repositioning and biological errors are small.

![Perfusion fluctuations in single voxels of white and grey matter over the course of a scan. Each data point is the measurement from a single average.](image)

**Figure 5.3:** Perfusion fluctuations in single voxels of white and grey matter over the course of a scan. Each data point is the measurement from a single average.

Across all tissue volumes the white matter has a lower absolute error than the grey matter. Figure 5.3 shows the perfusion fluctuations in single voxels of grey and white matter over the course of 45 averages (6 minutes). Fluctuations in grey matter have a higher amplitude, causing greater differences between the randomly separated averages, leading to larger absolute errors. This difference in fluctuation amplitude in different tissues is likely to be biological in origin. Grey matter has a higher neuronal density than white matter, requiring a more dense vascular network [48]. This gives scope for a greater amplitude of perfusion fluctuation in response to changes in neuronal activity. Section 5.4 investigates the temporal correlations in these fluctuations between neighbouring voxels. For a given volume size, percentage errors are however greater in white
matter due to the lower absolute value of white matter perfusion.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Tissue volume</th>
<th>Mean ± sd of difference data (number of samples)</th>
<th>95% confidence limit</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASL (Current study)</td>
<td>Whole brain</td>
<td>-0.02% ± 5.5% - unbiased (10)</td>
<td>7.6% ± 1.8%</td>
</tr>
<tr>
<td>PET (Matthew '93)</td>
<td>Whole brain</td>
<td>2.3% ± 8.7% - unbiased</td>
<td>12.0% ± 1.7%</td>
</tr>
<tr>
<td></td>
<td>Occipital Cortex</td>
<td>0.7% ± 11.9% - unbiased (25)</td>
<td>16.4% ± 2.4%</td>
</tr>
<tr>
<td>IMP-SPECT (Podreka '89)</td>
<td>hemisphere</td>
<td>3.8% ± 7.8% (estimated from graph) - unbiased (28)</td>
<td>10.8% ± 1.5%</td>
</tr>
<tr>
<td>CT (Gillard '01)</td>
<td>hemisphere</td>
<td>-0.1% ± 21.5% (estimated from graph) - unbiased (28)</td>
<td>29.7% ± 5.8%</td>
</tr>
<tr>
<td>USPIO Bolus tracking in pig (Simonsen '99)</td>
<td>Single slice</td>
<td>sd of 10% for five measurements in a single pig.</td>
<td>20%</td>
</tr>
</tbody>
</table>

Table 5.3: Published values of perfusion reproducibility, converted into 95% confidence limit. The bolus tracking data (last row) is not difference data but data from 5 repeated perfusion measurements, from which the sd of the perfusion measurement is found directly. IMP = isopropyl[^32]I-p-iodoamphetamine. USPIO = ultrasmall superparamagnetic iron oxide.

The reproducibility compares favourably with other scanning techniques (table 5.3). Reproducibility is quoted in many different forms, most commonly as a mean difference and standard deviation between scan re-scan or as a correlation coefficient. For all the publications on reproducibility that I found there was access to the original signed data, so it was possible to extract the unbiased standard deviation of the difference data. The results for PET [131], Bolus tracking [185] (using USPIO rather than Gd), SPECT [165] and CT [62] are shown in table 5.3. The CASL technique is more reproducible than all of the other techniques, based on the published values shown. SPECT and PET are comparable and not much worse than CASL, with Bolus tracking and CT the worse. The current implementations (eg [152]) of Bolus tracking may have improved reproducibility to the study shown here but no published values of reproducibility could be found.
5.2 Perfusion variation between normal subjects

It is important to measure the normal intersubject variation in perfusion in order to use the technique clinically. The variability gives an indication of the numbers of subjects that will be required to determine a significant difference in perfusion between two groups. Variations in perfusion for example with age and gender must be controlled for.

5.2.1 Methods

Scanning

Thirty-two subjects (19 female, 13 male, mean age 40.6 years, sd 14.3 years) were scanned using the scan parameters as described in section 4.1. Labelling time was 1.73 s, Delay time 0.75 s, slice thickness 6mm with 3mm gap, 45 averages. 3 image sets were collected for a $T_1$ measurement as described in section 4.5. This gave a total scan time of 9 minutes. The perfusion maps were calculated as described in the analysis section 5.1.1.

5.2.2 Results

5.2.2.1 Mean values

Mean perfusion over all subjects is shown in table 5.4 and figure 5.4 along with published measurements using other techniques. There is a wide spread in published values but the CASL measurements are generally in good agreement with other techniques.

The PET values using water as a tracer [116, 154] could well be underestimating true perfusion. These two studies did not take account of the restricted permeability of the capillary wall to water, which will cause an underestimation of perfusion of about 20% [86]. The higher perfusion values from the study by Herscovitch [86] are likely to be more accurate due to the higher permeability of butanol. A second problem with PET quantification comes from the large voxel sizes, typically larger than 10mm$^3$ [86, 154, 116]. This causes problems when trying to define regions of pure grey and white matter, producing an overestimation of white matter perfusion and an underestimation of grey matter perfusion. In my own work I have found the white matter estimate to be very dependent on the choice of segmentation. For example if a wider
<table>
<thead>
<tr>
<th>Technique</th>
<th>Age (yrs)</th>
<th>Tissue</th>
<th>Perfusion (mean ± sd)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASL (this study)</td>
<td>40.6 ± 14.3</td>
<td>White matter</td>
<td>24.2 ± 4.3</td>
</tr>
<tr>
<td></td>
<td>(n=32, 19F)</td>
<td>Grey matter</td>
<td>64.4 ± 12.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Whole brain</td>
<td>58.7 ± 9.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ratio GM/WM</td>
<td>2.68 ± 0.43</td>
</tr>
<tr>
<td>PET H215O</td>
<td>45.1 ± 15.2</td>
<td>White matter</td>
<td>22.2 ± 4.9</td>
</tr>
<tr>
<td>(Leenders '90)</td>
<td>(n=34, 16F)</td>
<td>Insular grey matter</td>
<td>54.5 ± 12.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frontal cortex</td>
<td>46.4 ± 10.7</td>
</tr>
<tr>
<td>PET butanol</td>
<td>34</td>
<td>White matter</td>
<td>38.5 ± 8.3</td>
</tr>
<tr>
<td>(Herscovitch '87)</td>
<td>(n=17, 8F)</td>
<td>Insular grey matter</td>
<td>69.8 ± 14</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Frontal cortex</td>
<td>61.8 ± 11.5</td>
</tr>
<tr>
<td>PET H215O</td>
<td>46 ± 15</td>
<td>White matter</td>
<td>24.7 ± 5.3</td>
</tr>
<tr>
<td>(Pantano '83)</td>
<td>(n=27, 8F)</td>
<td>Grey matter</td>
<td>47.7 ± 10.9</td>
</tr>
<tr>
<td>IMP-SPECT</td>
<td>25.1 ± 5.3</td>
<td>Whole brain</td>
<td>67.5 ± 10</td>
</tr>
<tr>
<td>(Podreka '88)</td>
<td>(n=14, 7F)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>133Xe-SPECT</td>
<td>33 ± 10</td>
<td>White Matter</td>
<td>59 ± 12</td>
</tr>
<tr>
<td>(Devois '86)</td>
<td>(n=97, 41F)</td>
<td>Grey Matter</td>
<td>72 ± 12</td>
</tr>
<tr>
<td>133Xe, 16 detectors</td>
<td>28.5 ± 5</td>
<td>Grey Matter</td>
<td>89.1 ± 7.3</td>
</tr>
<tr>
<td>(Naritomi '79)</td>
<td>(n=28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gd bolus MRI</td>
<td>42 ± 13</td>
<td>White matter</td>
<td>23.7 ± 4.9</td>
</tr>
<tr>
<td>(Screiber '98)</td>
<td>(n=11)</td>
<td>Grey matter</td>
<td>67.1 ± 16.3</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Hemisphere</td>
<td>38.8 ± 7.2</td>
</tr>
<tr>
<td>Gd bolus MRI</td>
<td>55.6 ± 11.5</td>
<td>MCA territory</td>
<td>58.2 ± 20.4</td>
</tr>
<tr>
<td>(Guckel)</td>
<td>(n=13, 3F)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.4: Published perfusion measurements.

$T_1$ range (0.5 - 0.9 s) is chosen the white matter perfusion estimates are increased by about 15%. This is likely to be due to the inclusion of some mixed voxels containing both white matter and grey matter. The rather high white matter values in the study by Herscovitch could be due to partial volume.

The Gd bolus MRI, SPECT and Xenon grey matter measurements are in reasonable agreement with each other and with the results of my study. The hemispheric perfusion from the study by Schreiber [178] is quite a lot lower than my whole brain value, but also lower than the value in the study by Guckel using the same technique. The white matter estimate in the study by Devois is much higher than all the other white matter values. They comment on this in the paper and suggest reasons why their
Figure 5.4: *Published perfusion measurements from table 5.4. The error bars represent the standard deviations of the measurements. Where no mean grey matter value is given, the mean of the two regional grey matter values is used. For the Bolus tracking studies the hemispheric and MCA territory values are taken to be representative of whole brain perfusion.*

value is erroneously high: the assumption of a global value for the partition coefficient (percentage of water in brain to blood) of 1.0 which they estimate could lead to a 40% overestimation of white matter perfusion; Compton scattering causing perfusion overestimation in low flow areas surrounded by high flow areas; partial volume effects, i.e. contamination of white matter voxels by grey matter.

The standard deviation of the mean values, representing the intrasubject spread in perfusion are also in agreement with published values. They show a fairly high variability in perfusion between different subjects.

### 5.2.2.2 Age effects

Figure 5.5 shows the grey and white matter perfusion measurements for all subjects, correlated with age. Figure 5.6 shows the perfusion ratio of grey matter to white matter with age. A straight line is fitted to the data. There is found to be a significant reduction of grey matter perfusion with age of 0.5% per year (p<0.05) but none for white matter. The most significant correlation was found for the grey/white matter perfusion ratio. The ratio was found to fall by 0.8% per year (p<0.001).
Figure 5.5: Grey and white matter perfusion measurements in 32 subjects correlated with age. The error bars represent the 95% confidence limit on the measurements.

Figure 5.6: Ratio of grey to white matter perfusion for 32 subjects correlated with age. The error bars represent the 95% confidence limit on the measurements.
<table>
<thead>
<tr>
<th>Technique</th>
<th>Tissue</th>
<th>% Perfusion change per year</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASL (n=32) (current study)</td>
<td>Grey matter</td>
<td>-0.50 (p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Whole brain</td>
<td>-0.43 (p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Ratio GM/WM</td>
<td>-0.80 (p&lt;0.001)</td>
</tr>
<tr>
<td>PET (n=34) (Leenders '90)</td>
<td>Insular Grey Matter</td>
<td>-0.47 (p&lt;0.05)</td>
</tr>
<tr>
<td></td>
<td>Frontal Cortex</td>
<td>-0.51 (p&lt;0.05)</td>
</tr>
<tr>
<td>PET (n=30) (Martin '91)</td>
<td>Limbic and association cortices,</td>
<td>-0.5 (p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>normalised to global flow</td>
<td></td>
</tr>
<tr>
<td>PET (n=26) (Pantona '84)</td>
<td>Grey matter</td>
<td>-0.7 (p&lt;0.02)</td>
</tr>
<tr>
<td>PET (n=20) (Bentourkia '00)</td>
<td>Whole Brain</td>
<td>-0.37 (p=0.035)</td>
</tr>
<tr>
<td>$^{133}$Xe SPECT (n=97)</td>
<td>Whole brain</td>
<td>-0.46 (p&lt;0.05)</td>
</tr>
<tr>
<td>(Devous '86)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{133}$Xe16 detectors (n=46)</td>
<td>Grey Matter</td>
<td>-0.63 (p&lt;0.001)</td>
</tr>
<tr>
<td>(Naritomi '79)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{133}$Xe 16 detectors (n=44)</td>
<td>Whole brain</td>
<td>-0.41 (p&lt;0.001)</td>
</tr>
<tr>
<td>(Melamed '80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{133}$Xe 16 detectors (n=15)</td>
<td>Grey Matter</td>
<td>-0.68 (p&lt;0.05)</td>
</tr>
<tr>
<td>(Meyer '78)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5: Published values of perfusion changes with age.

These results are in agreement with previous studies using other techniques as shown in table 5.5 and figure 5.7. In addition, none of the previous studies showed a significant correlation of white matter perfusion with age. White matter perfusion was generally found to remain steady with age, in agreement with my results. A study of cerebral blood volume changes [209] found a similar grey matter reduction of 0.6% per year, but also a 0.3% per year decline in white matter. Wide inter-subject variability in perfusion is seen, well beyond the 95% confidence interval on the measurements.
Figure 5.7: Published values of perfusion changes with age from table 5.5. For PET (Leenders) the mean of the two regional grey matter values is used.

Figure 5.8: Perfusion measurements in age-matched male (n=13, mean age 42.9 sd 12.3 years) and female (n=15, mean age 42.2 sd 15.1 years) volunteers. The error bars show the standard deviation between measurements. The significance of male to female differences are calculated using a t-test.

5.2.2.3 Gender effects

Figure 5.8 shows the gender difference in perfusion between two groups of age matched controls (13 male, mean age 42.9 sd 12.3 years; 15 female, mean age 42.2 sd 15.1 years). Females are found to have significantly higher grey matter (p=0.05) and whole brain
Table 5.6: Published values of perfusion differences with gender.

<table>
<thead>
<tr>
<th>Technique</th>
<th>Age range (yrs)</th>
<th>Tissue</th>
<th>% Perfusion increase in females</th>
</tr>
</thead>
<tbody>
<tr>
<td>CASL (current study)</td>
<td>25 - 67 (n=28, 15F)</td>
<td>Grey matter</td>
<td>15% (p=0.05)</td>
</tr>
<tr>
<td></td>
<td>Whole brain</td>
<td></td>
<td>16% (p=0.02)</td>
</tr>
<tr>
<td>133Xe-SPECT (Devoe '86)</td>
<td>20 - 29 (n=51, 16F)</td>
<td>Whole brain</td>
<td>10% (p=0.01)</td>
</tr>
<tr>
<td></td>
<td>30 - 39 (n=20, 8F)</td>
<td></td>
<td>12% (p=0.03)</td>
</tr>
<tr>
<td>IMP-SPECT (Podreka '89)</td>
<td>19 - 40 (n=14, 7F)</td>
<td>Grey matter</td>
<td>16% (p&lt;0.02)</td>
</tr>
<tr>
<td>CASL (Floyd '01)</td>
<td>20 - 38 (n=10, 5F)</td>
<td>Whole brain</td>
<td>40% (p&lt;0.005)</td>
</tr>
<tr>
<td>133Xe (Shaw '79)</td>
<td>20 - 29 (n=60, 18F)</td>
<td>Grey Matter</td>
<td>15% (p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>30 - 39 (n=34, 13F)</td>
<td></td>
<td>15% (p&lt;0.01)</td>
</tr>
<tr>
<td></td>
<td>50 - 59 (n=34, 17F)</td>
<td></td>
<td>8% (p&lt;0.05)</td>
</tr>
</tbody>
</table>

Figure 5.9: Published values of perfusion differences with gender from table 5.6. Where there is more than one age range, the mean perfusion increase over all age ranges is shown.

(p=0.02) perfusion; about 15% higher than in males. This is consistent with published results as shown in table 5.6 and figure 5.9. The study by Floyd [57] showed a larger effect than the other studies, a 40% (p<0.005) increase in female perfusion. This value could be unrepresentative of the general population due to the small number of subjects (10) in this study. The extensive study by Shaw [181] found the gender effect to be age-dependent with older females (age 50 - 59 years) showing a smaller effect. The
study by Devous [44] found female perfusion to be increased at all age ranges but the increase was larger and only significant for ages 20 - 39 years. I found I had too few data points to produce any significant differences within smaller age ranges. Interestingly, one study by Baxter [17] found a 19% increase in female glucose metabolism compared to males, which is expected to correlate with perfusion changes. However, two other studies of glucose metabolism [7, 141] found no gender effect.

5.2.2.4 Analysis of pooled age and gender data

<table>
<thead>
<tr>
<th>Tissue</th>
<th>% Perfusion Change per year</th>
<th>% Perfusion Increase in Females</th>
</tr>
</thead>
<tbody>
<tr>
<td>White Matter</td>
<td>0.35</td>
<td>8.4</td>
</tr>
<tr>
<td></td>
<td>p=0.13</td>
<td>p=0.22</td>
</tr>
<tr>
<td>Grey Matter</td>
<td>-0.43</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td>p=0.055</td>
<td>p=0.09</td>
</tr>
<tr>
<td>Whole Brain</td>
<td>-0.36</td>
<td>12.9</td>
</tr>
<tr>
<td></td>
<td>p=0.07</td>
<td>p=0.04</td>
</tr>
<tr>
<td>Ratio GM/WM</td>
<td>-0.77</td>
<td>5.7</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0005</td>
<td>p=0.19</td>
</tr>
</tbody>
</table>

Table 5.7: Pooled analysis of age and gender effects on perfusion. Significant results (p<0.05) are shown in bold.

Rather than analysing the effects of age and gender separately they can be pooled in a single analysis. This way the complete data set can be used for the gender analysis (rather than choosing only age-matched groups) and the effect of gender on the age dependence is taken into account. SPSS was used to perform univariate analysis with perfusion as the dependent variable, age as a covariate factor and gender as a fixed factor. The results (table 5.7) are similar to those from the separate analyses. The decrease in grey matter perfusion per year is slightly reduced (-0.43% compared to -0.5%) and is less significant (p=0.055). The decrease in GM/WM perfusion per year (-0.8%) remains highly significant (p<0.0005). Female perfusion is increased compared to males for all tissue types, but the increases are smaller and only significant for whole brain (12.9% increase compared to 16%, p=0.04). The results of this analysis do not affect the conclusions of this study.
5.2.3 Discussion

The results of this section have shown significant differences in perfusion between subjects and with age and gender. These variations may reflect true perfusion differences or could be due to other biological or instrumental effects causing artefactual changes to the perfusion estimates. Some of the published works discuss these issues, and my results can shed further light.

5.2.3.1 Grey matter reduction in perfusion with age

Non-perfusion biological changes

There are a number of biological changes with age that could cause an apparent but artefactual reduction in perfusion with age. Firstly, normal aging is accompanied by cortical atrophy [37]. As many authors point out [129, 154, 116] as the cortex thins the contamination of the grey matter voxels by white matter and CSF will increase, reducing the measured grey matter perfusion. This effect will depend on voxel size. The fact that the results are very consistent across many different techniques with different resolutions and different sensitivities to atrophy, suggests that atrophy is not the cause. Cortical atrophy is global but some studies show regional specificity [129] in perfusion changes, suggesting a cause other than atrophy.

Instrumental factors

Other factors affecting the CASL perfusion measurement, namely arrival time, capillary permeability, labelling efficiency and the T₁ of blood could all change with age. For example, a reduction in blood velocity in the major feeding vessels would reduce the labelling efficiency leading to an underestimation in perfusion. However, these factors, and likewise factors such as delay and dispersion and partition coefficient in PET [18] are very dependent on the technique used. An increase in capillary permeability for example would cause opposite changes in perfusion estimate with CASL (perfusion underestimate) and PET (perfusion overestimate). Again, the widespread consistency in results over different techniques suggests these factors are not the cause.

So if the perfusion decrease with age is real, what is the cause? One explanation is that although the perfusion reductions are real they are not necessarily a consequence of old age. The reduction may be due to cerebrovascular disease; the percentage of cerebral artherosclerotic lesions increase from the age of 30 [37]. One study [39] with very careful
health exclusions found no significant perfusion change with age. An indirect cause of reduced perfusion in older subjects could be reduced mental activity, for example due to reduced hearing and vision.

Normal aging is accompanied by decreases in synaptic density [90], neuron number [84] and neuronal size [195]. This could be a direct cause of perfusion reduction since reduced synaptic density will cause reductions in local metabolic need and therefore perfusion. Many studies [154, 116, 74, 224] show a reduction in the metabolic rate of oxygen and glucose with age, supporting the idea that the local metabolic demand is reduced. A study in children [34] showed temporal and spatial patterns of glucose utilisation correlated with synaptic density, thereby linking glucose metabolism with synaptic density. Capillary densities are also found to be higher in regions of high synaptic density [87].

The percentage decrease in cerebral cortex neurons with age from one study by Henderson [84] are 0.21% per year for glial cells, 0.57% for small neurons, and 0.73% for large neurons. Work by Anderson [5] confirms these findings but reports a slightly higher neuron loss of 1.0% per year. These values are in good agreement with percentage perfusion reduction with age. The variability in neuron number between individuals in Henderson's study also matches perfusion variations. For most individuals he found the cell numbers fall within 50% of the mean for that decade [84]. However there are some instances where numbers are 4 times greater in one individual than another. From figure 5.5 we can see that there are some instances where grey matter perfusion is twice as great in one person than another. Perfusion reductions could also be caused by diminished neuronal activity [129] (i.e. firing rate) rather than reduced numbers. This could cause similar changes to metabolic demand. It will be difficult to separate these two effects.

Numerous fMRI studies have shown perfusion increases during various cognitive tasks. It makes sense that the well known symptoms of old age - reductions in memory capacity, verbal performance and 'fluid intelligence' [129] should correlate with reduced perfusion. Interesting future work could identify if the grey matter perfusion reductions are global or localised to a certain region. This would shed more light on the possible cause of perfusion reduction.
5.2.3.2 Brain perfusion is higher in females

The gender effects have been less thoroughly discussed in the literature. These effects are less likely to be due to atrophy, or quantification factors such as labelling efficiency. A difference in T₁, between the sexes could explain the difference in the CASL measurements, but not the changes seen with non-MRI techniques. The differences could however be related to the brain volume supported by one study [116] which showed an inverse correlation between whole brain perfusion and brain size (p<0.01). Other suggested effects are hematocrit differences [165]; blood viscosity decreases [181] during menarche [132]; and increased oestrogen levels [17]. The smaller difference in perfusion values in older age supports the idea that it is due to some effect during menarche. Shaw [181] suggests that the continued increased perfusion in females in old age is due to oestrogen giving some protection against artherosclerosis. Recent work [133] supports the oestrogen hypothesis showing a grey matter perfusion reduction in female mice deficient in oestrogen receptors. The suggestion that the effect is due to the thinner female skull causing reduced photon attenuation in SPECT [165] is clearly incorrect due to the consistent results using different techniques. Very recent work [211] shows that females have a higher neuronal density than males in areas of the prefrontal and parietotemporal cortex of between 15% and 18%. Since metabolic demand and therefore perfusion are linked to neuronal density this could explain the perfusion gender differences.

In conclusion, large interindividual variability implies that comparisons with a patient group will only yield statistically significant differences if large numbers are considered. CASL should prove more successful in this area since it is difficult to get large numbers of volunteers to take part in an invasive PET study [116]. Age and gender effects must be corrected for in comparison studies.

5.3 Temporal stability

The previous section showed large variability in perfusion between individuals. This variation could be due to intrinsic differences in individual brains or it could simply reflect wide perfusion variation with time in each individual. In this section I aim to establish the temporal stability of perfusion within a single brain. Note that this is not a test of the stability of the measurement technique but of the stability of actual
biological perfusion. This information is important for clinical studies where the effect of disease or drug treatment on perfusion are measured over time. It is important to discount expected normal perfusion variations when interpreting this data. I believe the results should also be of physiological interest. I measured perfusion in one volunteer every hour over the course of a day and every day over the course of a week. Previously this has not been possible due to the invasive nature of perfusion measurements using PET and Gadolinium contrast MRI. To measure long term temporal stability, perfusion was measured twice in five subjects with a mean interval of 9 months.

5.3.1 Methods

Scanning
One volunteer (female, age 26) was scanned using the scan parameters as described in section 4.1. Labelling time was 1.73 s, Delay time 1.0 s, slice thickness 7mm with 2mm gap, 45 averages. 3 image sets were collected for a $T_1$ measurement as described in section 4.5. This gave a total scan time of 9 minutes. Scanning was repeated every hour over the course of a day for 8 hours, and every day (bar two) at approximately the same time for 8 days. Care was taken to reposition the subject at the same position in the scanner and to prescribe the slices in the same location. Notes were taken of food and drink consumption and if the volunteer fell asleep during scanning. The perfusion maps were calculated as described in the analysis section 5.1.1.

Long term temporal stability
This same protocol was used to measure perfusion in five volunteers twice (mean age 31.2 yrs at time of first scan, 2 female) with a mean time interval of 9 months.

To calculate if differences between measurements were significant I used the standard deviation for the unbiased difference data, given in table 5.1. Multiplying these values by 1.95 gives the 95% confidence interval on the difference data. These are given in table 5.8. If the measured change is greater than this, it is significant at the $p=0.05$ level.
Figure 5.10: *Perfusion variation in a single subject over the course of a day. The error bars show the 95% confidence limit of the measurements.*

### 5.3.2 Results

#### 5.3.2.1 Perfusion changes in a day

Figure 5.10 shows the results of serial perfusion measurements throughout one day. The error bars on the y axis show the 95% confidence interval on the measurements, taken from the reproducibility measurements. During the day the average perfusion value was 75 with a range of (67-80) ml blood min\(^{-1}\) (100ml tissue\(^{-1}\)) for grey matter, 29 (26-33) ml blood min\(^{-1}\) (100ml tissue\(^{-1}\)) for white matter and 67 (62-72) ml blood min\(^{-1}\) (100ml tissue\(^{-1}\)) for whole brain. Food was consumed at 13 hours and 17 hours.

There is a significant drop in grey matter perfusion at 15 hours which could be a consequence of food consumption two hours before. It is possible that digestion could divert blood to the stomach, reducing cerebral perfusion. This is perhaps unlikely given the effectiveness of perfusion autoregulation in the brain. The drop at 19 hours, also two hours following food consumption, supports this idea. However, perfusion would perhaps be expected to drop earlier after food intake than the 2 hour lag that I report. White matter shows lower absolute variability than grey matter, suggesting that perfusion is altered in response to changing metabolism and awareness, localised in the grey matter.
5.3.2.2 Perfusion changes in a week

Figure 5.11 shows the results of serial perfusion measurements throughout one week. During the week the average perfusion value was 72 with a range of \((64-84) \text{ ml blood min}^{-1} (100 \text{ ml tissue})^{-1}\) for grey matter, \((27 - 31) \text{ ml blood min}^{-1} (100 \text{ ml tissue})^{-1}\) for white matter and \((64 - 73) \text{ ml blood min}^{-1} (100 \text{ ml tissue})^{-1}\) for whole brain. The variation in perfusion values over one week are larger than those during one day. There is a significant drop in grey matter, white matter and whole brain perfusion on Monday. Could this be that ‘Monday morning feeling’?! The perfusion ratio of grey matter to white matter remains stable.

5.3.2.3 Long-term perfusion changes

Table 5.8 shows the results of the scanning at intervals of several months in five volunteers. Three subjects showed no significant perfusion changes. There are no significant changes to the perfusion ratio of grey matter to white matter in any subjects. The perfusion ratio also shows the lowest mean magnitude change.
Table 5.8: Percentage perfusion changes over several months measured in five individuals. The stars indicate significant changes in perfusion, where the differences were greater than the 95% confidence limit in difference measurements.

5.3.3 Discussion

Within the errors of the measurement, perfusion remains reasonably stable throughout a day and a week within one subject. There are significant variations in perfusion in two out of five subjects over a longer period of several months. The perfusion ratio of grey matter to white matter showed no significant changes in any subject over any time interval. This could be partly due to the poorer reproducibility of this measurement such that changes could not be accurately detected. The ratio is likely to be more stable because it will not be affected by global perfusion changes such as those due to food digestion or dehydration.

5.4 Spatio-temporal correlations within the brain

During the analysis of single pixel reproducibility I noticed that the temporal perfusion pattern of neighbouring grey matter pixels appeared to be correlated. i.e. over the course of the 6 minute scan if one voxel showed a sudden increase or decrease in one
of the perfusion averages, its neighbour had a tendency to do the same. I decided to investigate this further using a larger voxel size of 1.95 cm³ in order to boost the signal to noise. The voxels were cubic with length 12.5 mm. Perfusion weighted images were collected every 8 seconds for a period of 6 minutes. The correlation coefficient, C, is given by:

\[ C = \frac{\sum_{i=1}^{N} (x_i - \bar{x})(y_i - \bar{y})}{N \cdot \sigma_x \cdot \sigma_y} \] (5.1)

where \( x_i \) and \( y_i \) are the perfusion measurements in voxels \( x \) and \( y \) at time \( i \); \( \bar{x} \) and \( \sigma_x \) are the mean and standard deviation of the perfusion values in voxel \( x \) over all time points \( N \), and likewise for voxel \( y \). This was calculated between the signal from each voxel \( (x) \) and every other voxel in the same slice \( (y) \) over all time points \( (i) \). If perfusion in voxel \( x \) increases above the mean \( ((x_i - \bar{x}) \text{ positive}) \) at the same time as perfusion in voxel \( y \), then \( C \) will increase. High correlations were found between both neighbouring voxels and more distant voxels. Figure 5.12 shows the voxels from one particular slice that had a correlation coefficient of greater than 0.7 with one other voxel, which could be at any location. It can be seen that most of these voxels are located in the grey matter.

Correlations between the temporal signal of neighbouring voxel pairs outside the head were also investigated. Out of 380 voxel pairs the highest recorded correlation was 0.51. This suggests that the coefficients of over 0.7 found within the brain are significant. The mean correlation coefficient outside the head was not zero but 0.06. This could be due to slight smoothing of the data somewhere in the processing, however, all known filtering processes were turned off. This small correlation in the noise could be due to image artefacts which extend outside of the head.

Figure 5.13 shows the correlation between the three voxels shown in yellow, green and blue on figure 5.12. The perfusion fluctuations are well correlated with a frequency of about 0.03 Hz. On searching the literature it appears that this is a well-reported phenomenon.

5.4.1 Correlations in the brain

Several studies have reported slow, resting-state fluctuations of physiological measurements using BOLD [121, 223, 104, 36, 105, 142, 19, 122, 92] and PET [88]. Fluctuations between both neighbouring and long-range regions have been found to be correlated.
Figure 5.12: The voxels coloured red represent voxels that have a temporal correlation coefficient of greater than 0.7 with another voxel. The voxel coloured yellow has a correlation coefficient of 0.74 with the voxel coloured green and 0.77 with the voxel coloured blue. The image on the right shows the $T_1$ map of this brain slice.

Figure 5.13: The perfusion time course of the yellow, green and blue voxels from figure 5.12 are shown in corresponding colours.
All of the studies report fluctuations of less than 0.1 Hz.

One study [122] cites the importance of the temporal sampling rate. It is clear that

![Respiratory Fluctuations and Undersampled Signal](image)

Figure 5.14: **Undersampling of perfusion fluctuations.** Undersampling can produce artefactual low frequency signal, as shown by the dotted line.

at my slow sampling period of 8 sec the correlations could be due to aliasing of higher frequency physiological fluctuations such as heart rate and respiration. For example, figure 5.14 shows the result of sampling a signal of period 3s (typical respiratory time period) every 8 seconds. The measured signal shows artefactual fluctuations at a lower frequency.

Many of the studies conclude that the fluctuations are a manifestation of the functional connectivity in the brain. There is good evidence: the correlations are strongest between structures with known anatomical connections [223, 142, 19]; the correlations are decreased in the motor cortex of Multiple Sclerosis patients where there is damage to the anatomical connections [121]; and when using trans magnetic stimulation (TMS) on one cortical area, perfusion increases were observed in areas with known anatomical connections [161]. Work in the rat [69] has shown that perfusion fluctuations correlate with electrical activity. Other work [104, 105] suggests that the fluctuations might be due to blood pressure fluctuations brought about by changes in vessel diameter. The aim being to finely control the balance between blood flow and metabolism. Changes to the fluctuations have been observed during hypercapnia, when blood vessels are known to dilate [89]. These types of fluctuations might be contained within regions supplied by a single vessel.

As far as I am aware there have been no reports on low frequency perfusion fluc-
tuations measured using ASL. A better controlled experiment than mine with greater
temporal sampling would be interesting future work. This could be of benefit over the
BOLD studies in which a number of factors such as blood oxygenation, perfusion and
blood volume could all contribute to the fluctuations. ASL perfusion measurements
could help separate these effects.

5.4.2 Correlations in the heart and lungs

Parallel work on blood perfusion in the heart and lungs also reports local correlations in
perfusion. The focus here is not on temporal correlations but on correlations between
neighbouring regions at a single time point. In both the myocardium [15] and the lung
[67] it is found that on dividing the tissue ever more finely, more and more of the under­
lying heterogeneity is revealed. The heterogenous patterns of blood perfusion remain
the same on all length scales. A consequence of this observation is that the variance in
blood perfusion measurements will increase with increasing spatial resolution, while the
mean perfusion will remain the same. This causes the relative dispersion RD (RD =
standard deviation/mean) to increase with increasing resolution [15]. This relationship
can be linked to a fractal process [94] with fractal dimension D:

\[
\frac{RD(V)}{RD(V_0)} = \frac{V^{(1-D)}}{V_0}
\]  

(5.2)

where V is the voxel volume. Plotting ln(RD) versus ln(V) gives a straight line with neg­
ative slope 1-D. The steeper the slope, the larger the value of D. This fractal relationship
is found to hold true from volumes of several cm^3 down to the microscopic level [16, 65].

The value of D describes the strength of correlation between neighbouring voxels.
The correlation coefficient \( r_n \) between voxels n units apart is given by:

\[
r_n = \frac{1}{2}[(n - 1)^{2H} - 2n^{2H} + (n + 1)^{2H}]
\]  

(5.3)

where H=2-D [14]. This correlation coefficient is the same as that described in equation
5.1 except the summation is not over time but space. x and y are voxel pairs and the
sum is over all N voxel pairs in a given volume. The correlation is exactly the same
between neighbours of one unit size as between those of a smaller unit size. If D=1
the perfusion is uniform across all voxels (i.e. \( r=1 \)), if D=1.5 there is no correlation
(i.e. \( r=0 \)) and perfusion is random [15]. Measurements in the myocardium and lungs
give D values of 1.15 in the rat heart using ASL [16]; 1.21 in the canine heart using
microspheres [143] and 1.09 in the canine lung using microspheres [67]. I could find no reported measurements in the brain.

5.4.3 Measurement of spatial perfusion correlations in the brain

I decided to measure the relative dispersion and spatial correlations in brain perfusion measurements in order to test the fractal model.

5.4.3.1 Methods

MRI

One volunteer (female, age 26) was scanned using the scan parameters as described in section 4.1. Labelling time was 1.73 s, delay time 1.0 s, 8 slices, resolution 32 x 32, 45 averages. 3 image sets were collected for a T1 measurement as described in section 4.5. Six scans were acquired with varying slice thickness, slice gap and field of view, giving different voxel sizes. Cubic voxels of dimension 5 mm to 12.5 mm were chosen. By adjusting the slice gap, the slice centre position was kept constant for all scans. All known filtering of the images was turned off. The perfusion maps were calculated as described in the analysis section 5.1.1.

Relative Dispersion Analysis

The mean and standard deviation of the whole brain perfusion was calculated for each voxel size. Using RD = standard deviation/mean the relative dispersion RD was found.

One cause of increasing relative dispersion with decreasing voxel size could be the uncovering of more and more structure. If each structure has its own characteristic perfusion this will increase the relative dispersion. To check for this the analysis was repeated for white matter, which has a fairly homogenous structure. The white matter region was defined by manual segmentation from the T1 map. The analysis was also repeated on the T1 images in order to check that the correlations are due to the perfusing blood and are not present in other images.

It is possible that the increase in RD with decreasing voxel size is due to the reduction in signal to noise. To test this I collected a perfusion image with 180 averages. This data had voxel volume of 84 mm$^3$, smaller than the smallest voxel size of 125mm$^3$ used in this study. The whole brain and white matter RD was calculated for perfusion
maps generated using between 45 and 180 averages. The results in figure 5.15 show

Figure 5.15: The relative dispersion is independent of the number of averages (and therefore the signal to noise) of the perfusion measurement. The voxel size is 84mm$^3$, so the smallest number of averages here will have lower signal to noise than any of the measurements in this investigation.

there is no change to the relative dispersion with increased averages.

Spatial Correlation Analysis
For the smallest voxel size the correlation coefficient between nearest, next nearest, and next next nearest neighbours was found for whole brain and white matter perfusion. The perfusion $x_i$ was found for a set of $i$ voxels. The perfusion $y_i$ of a nearest neighbour was found for all $i$ voxels. The correlation coefficient $r_1$ was calculated as defined in equation 5.1, but this time $i$ is voxel count within a single image slice (not time). This was repeated with a set of next-nearest neighbours to give correlation coefficient $r_2$ and a set of next next nearest neighbours to give $r_3$. A value for $D$ was found by fitting equation 5.3 to the data. Correlations in the $T_1$ map and in the noise outside of the head were also investigated.

5.4.3.2 Results

Relative dispersion
Figure 5.16 shows the results of plotting $\ln(RD)$ against $\ln(V/V_0)$. Results are shown for whole brain and white matter values of both perfusion and $T_1$. From equation
Figure 5.16: Relative dispersion (RD) of perfusion and $T_1$ data as a function of voxel volume $V$. $V_0$ is the smallest voxel size of 125 $mm^3$.

The slope of this line is equal to $1-D$. For perfusion the fit gave $D=1.13$ (standard error (se) 0.02, $p<0.005$) for whole brain and $D=1.26$ (se 0.02, $p<0.005$) for white matter. Corresponding values for $T_1$ were $D=1.21$ (se=0.03, $p<0.005$) for whole brain and $D=1.09$ (se=0.03, $p>0.05$ i.e. not significant) for white matter.

Spatial correlation

Figure 5.17 shows the local spatial correlations of the perfusion and $T_1$ data. The fractal model, equation 5.3 fits well to the perfusion correlations.

5.4.3.3 Discussion

The relative dispersion results show that the perfusion data follows fractal behaviour, giving a value for $D$ similar to those found in the heart and lung [16, 143, 67]. However, the $T_1$ whole brain data also shows a reduction in RD with voxel size. This suggests that the effect is in part due to the structure of the brain. On dividing the tissue ever more finely we reveal more and more structure, increasing the RD of any measurement.
Figure 5.17: Spatial correlations in whole brain and white matter perfusion and $T_1$ as a function of distance. The fractal dimension $D$ was found by fitting equation 5.3 to the data.

This is especially true for perfusion where there is a large difference in perfusion between different structures. This is less of a problem in the heart and lung since they have more homogenous structures.

However, the white matter is more homogenous so the increasing RD in perfusion for smaller voxels is unlikely to be due to structure. In support of this the $T_1$ data shows a smaller RD value for white matter than whole brain, and a much smaller change in RD with voxel size, suggesting that there is little structure to the tissue.

The fractal model fits well to the local spatial correlations in perfusion. For white matter the value of the fractal dimension $D$ (1.25) is in excellent agreement with that found by the relative dispersion analysis (1.26). The spatial correlations in $T_1$ are not well fit by the fractal model. The high correlations in the white matter $T_1$ measurements are somewhat puzzling since the signal is expected to be randomly distributed here. No significant correlations (all below 0.1) were found in the noise outside the head in either the $T_1$ or the perfusion images. Further work using higher resolution images could look for correlations within smaller tissue volumes. Correlations within other homogenous structures could be investigated.

So what is the cause of the fractal relationship? The heart and lung literature
suggests two conflicting explanations. First, the perfusion fractality could be due to the underlying fractality of the vessel architecture [66, 203]. This is supported by a stochastic model of the microcirculation, predicting fractal characteristics to macrovascular perfusion [94]. Another view [10] is that the increasing variations in local perfusion on smaller and smaller scales suggest highly localised regulatory control. i.e. the tissue modulates the flow to meet local metabolic demand. Indeed, local variations in metabolic activity are well correlated with perfusion [76, 40]. One study [16] in the rat heart showed that during vasodilation the fractal dimension decreased, representing an increase in local correlations. Therefore the fractality appears to be a functional property, and not due to the underlying anatomy. This is supported by a study [143] in the canine heart showing change to local perfusion correlations during reduced perfusion pressure and applications of lidocaine and adenosine.

Both these explanations could also be true in the brain. It would be interesting to look at what factors might affect the fractal nature of perfusion variations in the brain.

5.4.4 Conclusion

The results of this section have confirmed both local and long-range spatio-temporal correlations of perfusion in the brain. Local spatial correlations in the white matter are well fitted by a fractal model. It is important to consider this when analysing resting state spatio-temporal correlations. Any local correlations may not be a consequence of synchronous electrical activity due to neural connections, but simply a consequence of the fractal nature of the vessel architecture.

5.5 Summary

- **Reproducibility** Whole brain perfusion measurements have a 95% confidence interval of 7.6% ± 1.8%, better than published measurements using other techniques.

- **Mean Values** Mean perfusion in 32 subjects is measured as 64 (sd 12) ml blood min⁻¹ (100ml tissue)⁻¹ in grey matter; 24 (sd 4) ml blood min⁻¹ (100ml tissue)⁻¹
in white matter and 59 (sd 10) ml blood min\(^{-1}\) (100ml tissue){-1} in whole brain. There is wide variability between subjects, as much as 100% for same sex subjects of similar ages.

- **Age Effects** There is a significant (p<0.05) reduction in grey matter perfusion of 0.5% per year.

- **Gender Effects** Females have significantly higher grey matter (p<0.05) and whole brain (p< 0.02) perfusion compared to males (an increase of about 15%).

- **Temporal Stability** Perfusion remains reasonably stable in a single subject throughout a day and a week. Over several months 2 out of 5 subjects showed significant perfusion changes. The ratio of grey matter to white matter did not change significantly in any subject over all tests of temporal stability.

- **Spatio-temporal Correlations** Temporal correlations in perfusion were found both between neighbouring and long range brain regions. Local spatial correlations are well fitted by a fractal model.
Chapter 6

Measurements of perfusion change

This chapter reports on the use of the ASL technique to measure perfusion change in disease and activation. There are examples of measurements in stroke, arteriovenous malformation, and during activation of the motor cortex. The last section presents the results of a more extensive study in Multiple Sclerosis.

6.1 Stroke - a comparison with gadolinium bolus tracking

Ethics approval was obtained for a preliminary study to compare the methods of arterial spin labelling and gadolinium bolus tracking in evaluating perfusion changes following stroke. However, at the time of writing only one subject (male, age 57) has been scanned. In this case the damage to the brain was minimal as can be seen in the diffusion-weighted scan, figure 6.1. As a result no perfusion deficits could be seen on either of the perfusion scans. Nevertheless, the images serve as a useful comparison between the two techniques.

6.1.1 Methods

6.1.1.1 ASL

One patient was scanned using the scan parameters as described in section 4.1. Labelling time was 1.35s, Delay time 1.0s, slice thickness 7mm with 2mm gap, 45 averages. 3 image sets were collected for a T₁ measurement as described in section 4.5. The perfusion maps were calculated as described in the analysis section 5.1.1.
Figure 6.1: Diffusion-weighted image showing the area of reduced diffusion in the infarcted area as hyperintense. TE = 40ms, b factor = 1000 s mm$^{-2}$.

6.1.1.2 Bolus tracking

A standard clinical protocol was used. A dose of 10ml of 0.5M (equivalent to 0.05mM/kg) Gadolinium DTPA (Magnevist by Schering) was injected over 5 seconds followed by 20ml of saline chaser. Gradient echo EPI began on injection and continued for 2mins 46s. Scan parameters were: GE EPI, flip angle 90°, 4 slices, 7mm thick, 2mm gap, matrix 128x128, field of view 24 cm, TR 1.3s, TE 40ms. The 4 slices were located in the same position as the central ASL slices. The images were analysed using software provided by Leif Ostergaard [152]. For each slice an arterial input function was found by averaging the signal curve from a few voxels located in or around major feeding vessels. The residue function was found by deconvolving the arterial input and tissue signal curves, using the technique of single value decomposition. The perfusion values are not absolute, only relative.

The perfusion measurements were compared in 34 regions of interest from the four slices. Large regions of between 2cm$^2$ (small grey matter region) and 120cm$^2$ (whole slice) were chosen in specific anatomical locations that could be identified on both scans.
Figure 6.2: Perfusion images using Gadolinium DTPA bolus tracking (left) and CASL (right). The CASL image has been smoothed to facilitate visual comparison. The top row shows images in the same slice location as the diffusion weighted scan, figure 6.1. There is no obvious perfusion difference in the location of the infarct. High signal is seen in large vessels on the Gadolinium images.

6.1.2 Results

Figure 6.2 shows the perfusion maps produced by the two techniques. The ASL image has been smoothed to facilitate visual comparison. It can be seen that the Gadolinium image shows more detail due to the improved resolution, but also shows high signal from the major vessels. Large vessel signal is suppressed on the ASL image through the use of a delay time of 1s.

Figure 6.3 shows the correlation between the measurements from the two techniques. A straight line fit produces a correlation coefficient of 0.32 (p<0.001). This is improved
Figure 6.3: Correlation between perfusion measured by the two techniques of CASL and Gadolinium-DTPA bolus tracking. Perfusion is compared in 34 regions taken from 4 brain slices from one patient. The correlation coefficient of 0.33 is improved to 0.62 if the 5 largest values (shown inside the marked circle) from the bolus technique are excluded.

to 0.61 (p<0.001) if the higher perfusion values are excluded. These higher values appear to be either overestimated by the bolus tracking technique or underestimated by ASL. This difference could be due to the inclusion of large vessel signal with the Gadolinium technique. Since the tracer is intravascular there is no means of removing the contribution from large vessels. The overestimation may be reduced with the use of spin echo EPI instead of gradient echo EPI when the signal change is weighted towards smaller vessels. A comparison of bolus tracking with PET also found the bolus tracking technique overestimated perfusion in large vessel areas [150].

6.2 Arteriovenous Malformation

Arteriovenous malformations (AVMs) are congenital abnormalities of blood vessels. The blood is shunted between arteries and veins without perfusing the tissue. It is difficult to detect this reduced perfusion with Gadolinium bolus tracking due to the high signal from the large shunting vessels [38]. CASL may be a more successful technique since an increased delay time could allow the shunting blood to pass through, leaving only perfusing blood.
6.2.1 Methods

One patient (female, age 49) with a large AVM was scanned using CASL at a range of 7 delay times from 0 to 1.5s. Other scan parameters were labelling time 1.73s, slice thickness 7mm with 2mm gap and 45 averages. 3 image sets were collected for a T₁ measurement as described in section 4.5. The perfusion maps were calculated for each delay time as described in the analysis section 5.1.1. A high resolution, fast spin echo image (TR=2.2s, TE=84ms) was also collected in order to determine the location of the AVM.

6.2.2 Results

![Perfusion images of arteriovenous malformation](image)

Figure 6.4: Quantitative CASL perfusion images of arteriovenous malformation at increasing delay time.

Figure 6.4 shows the perfusion maps from one slice with increasing delay time. Two large abnormal areas with very high ‘perfusion’ are seen on the zero delay time image; one at the bottom (posterior of brain) and one on the right (left side of brain).

The magnetic resonance angiography (MRA) images in figure 6.5, taken 10 months earlier, clearly show the abnormal vessel formations in the brain. There are two regions of AVM, corresponding to the regions of high perfusion. The image on the right, in
Figure 6.5: 3D time of flight MRA images showing the abnormal vasculature in two AVMs. The image on the left is a maximum intensity projection in the axial plane, showing all the major vessels in the brain. The image on the right shows the mean of 10 raw MRA images chosen to correspond to the slice location of the perfusion images in figure 6.4. The slice is located through two large draining veins of the posterior AVM, and through large vessel shunts of the second AVM.

The same slice location as the perfusion images in figure 6.4, shows large draining veins from the back end of an AVM in the posterior region (bottom of image), and large vessel shunts from a separate AVM on the left hand side of the brain (right hand side of image). The fast spin echo image from the same slice location (figure 6.6) also shows abnormal vasculature in the same two regions.

Three regions of interest were outlined (see figure 6.6), two over the AVMs and also a third, normal tissue region. The mean perfusion in each of these regions was found at each delay time. The results are shown in figure 6.6. With increasing delay time the perfusion in region 2 returns to roughly the same value as region 3 on the contralateral side. As predicted, the increased delay time has allowed the non-perfusing blood in the arterio-venous shunt to pass through the tissue, leaving a measure of the underlying perfusion. In this case, the tissue perfusion appears normal. It is interesting to note the striking resemblance between the data from region 2 and the many-vessel model behaviour, back in Chapter 3, figure 3.16. The data follow a curve very similar to that
Figure 6.6: The FSE image at the same slice location as the perfusion images in figure 6.4. Areas of AVM can be clearly seen in regions 1 and 2. Region 3 contains normal-appearing tissue. The graph shows the perfusion measurements in these three regions with increasing delay time.

predicted for a region containing an arteriole of velocity 2cm/s. This supports the idea that the signal drop is due to blood in the large shunting vessels flowing straight through and out of the voxel. Region 1 shows different behaviour. Figure 6.6 shows the blood to this region is delayed, with blood continuing to arrive up to 1s delay time, indicated by the rising signal. At a delay time of 1.5 s the signal is still high, presumably from blood in large vessels. This shape of signal curve is what would be expected for draining veins at the back end of an AVM, which are found in this region as shown in the right hand image of figure 6.5. The arrival time is shorter, and the signal higher, than would be expected for normal veins due to the very fast passage of blood through the AVM shunt.

In conclusion, ASL could be a useful technique in AVMs. It can determine underlying tissue perfusion in regions with large shunting vessels, and can also detect blood arrival in large draining veins. A more extensive study would be interesting.
6.3 Motor activation

There has been a lot of recent interest in functional MRI (fMRI), the term given to measurements of physiological change during brain activity. Most applications use the Blood Oxygenation Level Dependent (BOLD) [149, 148] technique which is sensitive to changes in deoxyhaemoglobin concentration, detected using T₂*-weighted sequences. However, it is also possible to measure activation in terms of a perfusion change [43, 216]. During brain activation, changes to blood oxygenation, oxygen extraction fraction, oxygen metabolism, perfusion and blood volume can all change the BOLD signal [78]. Perfusion-based fMRI is advantageous since it depends only on a single physiological parameter, perfusion, leading to an easier interpretation and understanding of the results. The BOLD signal originates largely from the draining veins, where the deoxyhaemoglobin is most concentrated. Standard BOLD studies often show apparent activation over distant draining veins. With perfusion-based fMRI the signal is more closely related to the microvasculature [123, 110, 47] and hence better represents the location of neuronal activation. In addition, BOLD contrast must be sampled using susceptibility weighted images resulting in signal loss in important brain regions which have high static susceptibility such as orbitofrontal cortex and the inferior temporal lobes.

There is current interest in improving the temporal resolution of the ASL-fMRI techniques [219]. Clearly, the CASL technique as I have implemented it with a temporal resolution of 8s is not ideal for fMRI. However, given the relatively few studies using ASL I felt it would be interesting to see if I could identify areas of perfusion increase during a motor task. Perfusion increases on the order of 50-70% are expected [226, 229, 110].

This increase in perfusion could provide a good test for the two-compartment model. At this increased perfusion the model predicts an increase in the outflow effect (see Chapter 3), i.e. the signal loss due to the outflow of un-exchanged labelled spins will increase. Therefore, a 50% increase in perfusion will give less than a 50% increase in the difference signal (the distributed forms of equations 3.23 and 3.24 are non-linear in perfusion). At increasing delay times the outflow effect becomes more dominant and, for the same perfusion increase, there is a fall in difference signal increase. However, the
single compartment model predicts a linear increase in difference signal with increasing perfusion (equations 3.7 and 3.8 are linear in perfusion).

6.3.1 Methods

One subject (female age 26 years) was scanned using CASL at a range of 6 delay times varying from 0.25 to 1.5s. Other scan parameters were Labelling time 1.73s, slice thickness 7mm with 2mm gap and 30 averages. For each delay time the subject was at rest for the first 15 averages (2min), named the OFF period. For the final 15 averages, the subject engaged in finger tapping (sequential finger to thumb opposition), the ON period. Three image sets were collected for a $T_1$ measurement as described in section 4.5. The difference images (label - control) were calculated for the ON and OFF periods at each delay time. The OFF difference image was subtracted from the ON difference image to identify areas of activation.

6.3.2 Results

Figure 6.7: Regions of motor activation from perfusion subtraction images. Four regions of motor activation can be seen in two slice locations of the ON - OFF images. The $T_1$ maps show that these regions are located in the motor cortex.

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Figure 6.8: Perfusion-weighted difference signal from the 4 activated regions during rest (OFF) and activation (ON) with increasing measurement time. The regions are identified on figure 6.7. measurement time = labelling time + delay time + image time. The image time is different for each slice (see section 4.1 in Chapter 4)

Figure 6.7 shows 2 slices of the activation image (ON - OFF) for the delay time of 0.25s. Using the corresponding T1 map as an anatomical reference, areas of activation can be identified in the motor cortices. Figure 6.8 shows the signal time curves for these 4 areas of activation. In all areas for all delay times the signal is increased in the ON image.

6.3.2.1 Signal increase at increasing delay time

Predicted behaviour

The model equations from Chapter 3 can be used to predict the signal increase ON-OFF at increasing delay times. The single compartment model (equations 3.6, 3.7 and 3.8), and the distributed and slow two-compartment models (equations 3.23 and 3.24) were considered. The difference signal $\Delta M$ was calculated at two perfusion rates, 60 and 90 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$, at a range of delay times. Fixed model parameters were: $PS=1.5$ ml water min$^{-1}$ (ml tissue)$^{-1}$, $v_5=0.05$, $v_w^p=0.7$, $t_A=0.8$ s, $t_L=1.7$ s, $T_{1e}=1$ s, $T_{1b}=1.4$ s and $\alpha=0.7$. Figure 6.9 shows the percentage increase in the difference signal at the higher perfusion rate (called ON-OFF) for each of the models. The single compartment and slow two-compartment models predict that the percentage
Figure 6.9: Predicted increase in ON - OFF difference signal with increasing measurement time. The distributed model predicts a fall in signal due to increased outflow of labelled spins during the high perfusion ON period. measurement time = labelling time + delay time.

Figure 6.10: Measured increase in ON - OFF difference signal falls with increasing measurement time. The data is averaged over all 4 regions as defined on figure 6.7. measurement time = labelling time + delay time + image time.

signal increase will be independent of delay time. The percentage increase of 50% is the same as the percentage increase in perfusion. The distributed model predicts a steady fall in the percentage signal increase with increasing delay time. This is because, at the higher perfusion rate of 90 ml blood min$^{-1}$ (100 ml tissue)$^{-1}$, the signal loss due to the outflow of un-exchanging labelled spins becomes significant. This effect increases with delay time as more labelled spins have time to exit the voxel. The percentage signal increase falls below the percentage perfusion increase.
Measured behaviour

Figure 6.10 shows the measured signal change (ON - OFF) with increasing measurement time, averaged over the 4 regions of activation. It can be seen that the difference signal does indeed fall with increasing measurement time, with results very similar to that predicted by the distributed model. Assuming the perfusion change is the same at all delay times this suggests that the distributed model is accurate. i.e. the outflow effects are coming into play at higher perfusion rates.

6.3.2.2 Quantitative perfusion increases

The data at the shortest delay time of 0.25s is least affected by the outflow of labelled spins, as shown by figure 6.9. The simplified slow solution (equations 3.22, 3.23 and 3.24) can be used to estimate the increase in perfusion. Averaged over all 4 regions I measure an increase in perfusion of 57% during motor activation, in good agreement with other studies [226, 229, 110].

6.3.3 Discussion

It is possible that a number of other factors could cause this drop in raw signal increase. First, one study [73] by Gonzalez-At et al. has shown a reduction in arrival time ($t_A$) during activation. Simulations show that, for measurement time $t < t_L + t_A$ this causes a fall in percentage signal with measurement time with the single compartment and slow solution. However, for measurement times greater than this the percentage change does not fall with measurement time. In the current study at measurement times greater than 2.5s figure 6.8 shows that the signal curves are falling, indicating $t > t_L + t_A$. Figure 6.10 shows that the percentage signal change continues to fall after this time. Interestingly, the study by Gonzalez-At et al. [73] also showed a reduction in percentage signal change with delay time, but attributed this to arrival time change. Another possibility is that actual, biological perfusion increase is falling with delay time. Since the measurements were taken in order from shortest to longest delay time it is possible that habituation to the task caused a reduction in brain activation at the longer delay times. Finally, the sensitivity to larger vessels at the shorter delay times could cause a difference. If the signal increase due to larger vessels is proportionally greater than that in smaller vessels there could be a reduction in the percentage signal change with increasing delay time.
6.4 Multiple Sclerosis

Multiple sclerosis (MS) is a chronic inflammatory disease of the central nervous system in which there are multiple areas of demyelination within the brain and spinal cord. The disease involves focal disruption of the blood-brain barrier with associated immune response and myelin damage. The key factors triggering the inflammatory and demyelinating processes are uncertain. There are four patterns of the disease: relapsing and remitting (RR), benign (B), secondary progressive (SP) and primary progressive (PP). Relapsing and remitting patients suffer a series of clinical episodes, each of which resolve with time. Benign patients have minimal disability after 10 years of first diagnosis. Secondary progressive patients suffer relapses and remissions but with underlying progressive disability. Primary progressive patients suffer increasing disability without relapses. MRI has been useful in the diagnosis and understanding of MS but correlations between MRI abnormalities and disability are poor [140]. Recent interest in abnormalities in the normal appearing brain tissue [55] may improve our understanding of the disease.

There are two reasons why perfusion might be a useful measurement in MS. First, perfusion is an indicator of tissue metabolic function. Therefore, in chronic lesions with irreversible damage, perfusion deficits are predicted [91]. This functional measure could correlate better with disability than the current structural MRI measures. Secondly, perfusion is expected to increase in areas of vascular inflammation due to the release of vasoactive substances [91]. Inflammation occurs in acute lesions as a precursor to demyelination and may be a primary event in the evolution of the disease [82].

To date there have been only a handful of studies measuring blood perfusion in MS using SPECT [124, 166] and PET [28, 191]. In addition there are three studies reporting cerebral blood volume changes measured using MRI bolus tracking with Gd-DTPA [82, 91, 164] and several studies on glucose metabolism [22, 21, 159, 171, 9, 176]. The four perfusion studies using PET and SPECT all showed a reduction in grey matter perfusion. However, these techniques all have poor spatial resolution giving non-pure grey matter voxels susceptible to partial volume effects with white matter and CSF. MS is often accompanied with grey matter atrophy [8] which would increase these partial volume affects and cause an apparent reduction in perfusion. The measured reduction
could be due to decreased grey matter perfusion, or volume, or both. Two of the studies showed correlations between regional reductions and clinical dysfunction [124, 166], and two [124, 191] showed perfusion reductions in areas of white matter. Two of the blood volume studies [91, 164] measured a reduction in the blood volume of both chronic and acute white matter lesions compared to normal appearing white matter. The more extensive study by Haselhorst [82] showed an increased blood volume in acute lesions compared to both normal appearing white matter and chronic lesions. All studies of glucose metabolism showed a reduction in metabolism in various grey matter regions, and one study [176] showed an increase in white matter lesion metabolism.

This study using ASL could provide a more accurate measurement of true capillary perfusion, and better spatial resolution compared to SPECT and PET.

6.4.1 Methods

34 MS patients (20 female, 14 male, mean age 49.9 years, sd 11.6 years) and 32 normal controls (19 female, 13 male, mean age 40.6 years, sd 14.3 years as in section 5.2) were scanned using the CASL technique as described in section 4.1. Scan parameters were Labelling time 1.73s, delay time 0.75s, 8 slices with 6mm thickness and 3mm gap, 45 averages. 3 image sets were collected for a T\textsubscript{i} measurement as described in section 4.5. The T\textsubscript{i} images were registered to the perfusion images to correct for any head motion, using a rigid registration technique as described in section 5.1.1. The perfusion maps were calculated as described in the analysis section 5.1.1.

White matter regions of interest were segmented automatically using T\textsubscript{i} values of 0.5 - 0.8s to include only relatively pure, normal appearing white matter. This will avoid most of the white matter lesions which have T\textsubscript{i} values in the range 0.7s to 1.6s [27] depending on lesion type. The automatic grey matter segmentation could not be used since there is a danger of misclassifying the white matter lesions as grey matter. Instead, cortical grey matter was manually segmented using the T\textsubscript{i} map. The ratio of grey to white matter perfusion was also recorded. In general, lesions were too small to get a reliable measurement of lesion perfusion.

For each subgroup of MS, age and gender-matched normal controls were chosen in order test for significant perfusion changes using a paired t-test. A group of age-
matched and gender matched controls was chosen to test for significant changes in the whole MS cohort (t-test, 2 sample unequal variance).

6.4.2 Results

![Graph](image)

**Figure 6.11:** Mean Grey and White Matter perfusion in 32 normal subjects and 34 MS patients, and each MS subgroup: relapsing remitting (RR), secondary progressive (SP), primary progressive (PP) and benign (B).

Figure 6.11 shows the white and grey matter perfusion values for the group of 32 normals and 34 MS patients and the MS subgroups. It can be seen that there is a trend to increased white matter and decreased grey matter perfusion in patients with MS. Secondary progressive patients show the greatest increase in white matter perfusion and primary progressive patients the greatest decrease in grey matter perfusion.

Table 6.1 shows the significance of the perfusion differences. There are significant increases in white matter perfusion for the relapsing remitting and secondary progressive subgroups compared to age and gender-matched normal controls. The secondary progressive subgroup also shows a significant decrease in the ratio of grey matter to white matter perfusion.

6.4.3 Conclusions

The greatest inflammatory activity in MS is likely to occur in the relapsing remitting subgroup, with least activity in benign and primary progressive subgroups. This matches the significance of the white matter perfusion increases, suggesting this increase could be linked to the inflammatory process. This is the first study to show significant
<table>
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<tr>
<th>Group</th>
<th>Age (yrs) mean ± sd</th>
<th>White Matter</th>
<th>Grey Matter</th>
<th>Ratio GM/WM</th>
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<td><strong>All MS</strong></td>
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<tr>
<td>Patients (n=34)</td>
<td>49.9 ± 11.6</td>
<td>28.0 ± 5.5</td>
<td>56.3 ± 9.7</td>
<td>2.05 ± 0.38</td>
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<td>Normals (n=20)</td>
<td>47 ± 12.7</td>
<td>24.7 ± 4.9</td>
<td>57.5 ± 10.6</td>
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<td><strong>p=0.67</strong></td>
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<tr>
<td></td>
<td></td>
<td>28.0 ± 5.5</td>
<td>56.3 ± 9.7</td>
<td>2.05 ± 0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td><strong>p=0.03</strong></td>
<td><strong>p=0.67</strong></td>
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<tr>
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</tr>
<tr>
<td>Patients (n=10)</td>
<td>39.1 ± 13.3</td>
<td>29.0 ± 4.9</td>
<td>61.9 ± 11.1</td>
<td>2.18 ± 0.45</td>
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<tr>
<td>Normals (n=10)</td>
<td>39.1 ± 13.2</td>
<td>22.9 ± 2.1</td>
<td>56.1 ± 9.4</td>
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<tr>
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<td>29.0 ± 4.9</td>
<td>61.9 ± 11.1</td>
<td>2.18 ± 0.45</td>
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<td></td>
<td><strong>p=0.005</strong></td>
<td><strong>p=0.18</strong></td>
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<td><strong>SP</strong></td>
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<td>Patients (n=5)</td>
<td>52.7 ± 8.6</td>
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<td>55.2 ± 7.3</td>
<td>1.82 ± 0.33</td>
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<td>Normals (n=5)</td>
<td>54.3 ± 11.7</td>
<td>24.7 ± 1.7</td>
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<td>31.2 ± 6.4</td>
<td>55.2 ± 7.3</td>
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<td><strong>p=0.04</strong></td>
<td><strong>p=0.33</strong></td>
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<tr>
<td><strong>PP</strong></td>
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<tr>
<td>Patients (n=11)</td>
<td>55.0 ± 8.9</td>
<td>26.2 ± 5.0</td>
<td>51.7 ± 9.1</td>
<td>2.01 ± 0.41</td>
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<td>Normals (n=11)</td>
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<td>56.1 ± 10.2</td>
<td>2.26 ± 0.33</td>
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<tr>
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<td></td>
<td>26.2 ± 5.0</td>
<td>51.7 ± 9.1</td>
<td>2.01 ± 0.41</td>
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<td></td>
<td></td>
<td><strong>p=0.04</strong></td>
<td><strong>p=0.18</strong></td>
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<tr>
<td><strong>B</strong></td>
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<td></td>
</tr>
<tr>
<td>Patients (n=8)</td>
<td>54.6 ± 4.1</td>
<td>27.3 ± 6</td>
<td>56.3 ± 7.8</td>
<td>2.1 ± 0.25</td>
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<tr>
<td>Normals (n=8)</td>
<td>55.3 ± 2.2</td>
<td>25.4 ± 3.3</td>
<td>56.6 ± 8.9</td>
<td>2.24 ± 0.29</td>
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<td><strong>p=0.45</strong></td>
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Table 6.1: Mean Grey and White Matter perfusion values (ml blood min⁻¹ (100 ml tissue)⁻¹) in 34 MS patients and each MS subgroup. For the subgroups perfusion values from age and gender-matched controls are used for a paired t-test to test the significance of the perfusion differences. Significance in the whole MS cohort is tested using 2 sample unequal variance t-test. Significant changes (p<0.05) are shown in bold.

Perfusion increases in the normal appearing white matter. However, it follows on from the finding of increased blood volume [82] and metabolic activity [176] in acute lesions. It is possible that perfusion is increased in tissue where new lesions are about to form as well as in the newly formed lesions. A serial study following perfusion changes in the development of new lesions would be of interest.

I found the greatest reduction in grey matter perfusion in the primary progressive group. This subgroup typically has more chronic lesions and the most disability, predicted to correlate with reduced perfusion. Further work looking at grey matter perfusion deficits in specific brain regions may show more conclusive grey matter results, in line with previous studies.

It is important to consider any other factors which might cause the white matter...
perfusion changes. One possible factor is the increased T1 of white matter in MS [27] of about 10% [75]. T1 is measured and so it should not affect the perfusion measurements, however it could have an effect on the segmentation. In order to define the same regions of white matter it might be necessary to use a larger upper limit (perhaps 0.85 rather than 0.8s) in the MS brain, or to manually segment. However, a larger upper limit would further increase the MS white matter perfusion estimates, due to increased partial volume with grey matter.

Another factor is the increased permeability of the vessels to water in MS [198]. In the modelling I assume the same fixed value of permeability for all of the analysis. An increase in permeability will cause more of the labelled spins to move into the extravascular space where they will lose their label more quickly. The signal would be lower than the model predicts, causing perfusion to be underestimated. Corrections for increased permeability will further increase the MS white matter perfusion values. Other factors such as arrival time, T1 of blood and the labelling efficiency would not be expected to be different between MS patients and normals.

So increased T1 or PS in the white matter of the MS patients would tend to mask perfusion increases, not give artificially high perfusion. Corrections for T1 and PS differences will increase the white matter perfusion differences between MS patients and normal controls.

### 6.5 Conclusion

This chapter has shown the results of several applications of CASL to measuring perfusion change. The examples in stroke and motor activation serve as useful validations of the technique and the modelling. The technique can detect both gross perfusion abnormalities as in the case of AVM, but also more subtle white matter changes as shown in the MS study.
Chapter 7

Conclusions and future work

7.1 Modelling

In this work I have implemented and tested the technique of Continuous Arterial Spin Labelling. The modelling of Chapter 3 addressed some of the remaining issues of quantification. The focus was on the permeability of the capillary wall to water. A two-compartment model was introduced which highlighted two opposing effects of using a realistic capillary permeability. Firstly there is a $T_1$ effect due to the difference in $T_1$ between blood and tissue. If ignored, this causes an overestimation of perfusion, especially in white matter. Secondly, the outflow effect, describing the outflow of unexchanged labelled water spins, leads to an underestimation of perfusion if ignored. Simplifications to the model produce solutions with fewer free parameters which can be used in vivo to measure perfusion.

7.1.1 Future work

There are a number of remaining inaccuracies in the two-compartment model.

- The model assumes that the blood is a well-mixed compartment. In reality there will be a concentration gradient of labelled spins from arterial to venous end. This leads to errors in setting the value for the venous magnetisation $\Delta m_v$. However, comparing the simulation results with those of St Lawrence et al. [189] (who did not make this assumption), suggests the error is small in human imaging at 1.5T. A more detailed comparison at other perfusion rates and field strengths would be interesting and useful.
• The model assumes labelled spins enter and leave the tissue voxel in exchanging vessels. The addition of arterial and venous compartments could be a more realistic model. St Lawrence et al. [189] has investigated the inclusion of a venous compartment and Alsop et al. [4] an arterial compartment, showing that they both have a substantial effect on the signal curve. Instead, the interpretation of PS as an average value for all of the vessels in the voxel could by-pass the need for these extra compartments. This could be investigated further.

• A further improvement to the model would be to include the MT effects on the extravascular T1. Two values of $T_{1e}$ could be introduced: $T_{1e} = T_{1\text{sat}}$ for $t \leq t_L$ and $T_{1e} = T_{1\text{unsat}}$ for $t > t_L$. $T_{1\text{sat}}$ and $T_{1\text{unsat}}$ are the T1 values in the presence and absence of the labelling pulse.

• The effect of different $T_2$ (or $T_2^*$) values for the blood and extravascular compartments could be incorporated into the model.

7.2 Validation and new measurements

By collecting data at a range of delay times it was possible to compare the accuracy of the different models. The simplified two-compartment solutions were found to be the most accurate over all extremes of perfusion data. The new model opened up the possibility of arrival time and permeability measurements as well as perfusion, as described in Chapter 4. The permeability parameter $PS/\nu_{bw}$ was found to be larger in grey matter than in white matter. This could be due to a difference in the location of the labelled spins at the time of measurement, i.e. in white matter the spins have not yet reached the capillaries. Arrival time was found to be linear with distance from the labelling plane. A further validation of the model was found in the motor activation study described in Chapter 6. The percentage increase in signal during activation dropped with measurement time, as predicted by the distributed two compartment solution. This suggests that the simplified slow solution will be inaccurate at higher perfusion rates, such as those during activation. Chapter 5 gives important reproducibility data to allow the technique to be used as a scientific tool. The reproducibility compared favourably with other perfusion techniques.
7.2.1 Future work

There are various additional methods of testing the accuracy of the model.

- Validation of the model for the spin labelling technique of FAIR is important. It is predicted that the impermeable solution will be accurate here, which would remove the need for a measurement of $T_{1e}$.

- The application of mild diffusion gradients [230] could remove the signal contribution from the larger vessels. It would be interesting to see if the apparently low white matter values of $PS/v_{bw}$ are increased as the signal comes from more permeable vessels.

- Measurements of muscle perfusion could test the accuracy of the model in a situation of increased $PS/v_{bw}$ (due to the lack of BBB). The single compartment model is predicted to be more accurate here.

- The models all predict different signal changes depending on the value of $T_1$ in blood and tissue. These predictions could be tested by altering the $T_1$ of blood or tissue through the use of a contrast agent.

7.3 Normal physiology

Results in a group of 32 normal subjects show high variability in perfusion measurements between subjects but reasonably stable perfusion within a subject over time. Grey matter perfusion is found to decrease with age by 0.5% per year and females have roughly 15% higher perfusion than males. Temporal correlations in perfusion were found between both neighbouring and distant voxels. Spatial correlations in perfusion between neighbouring pixels follow a fractal model.

7.3.1 Future work

- It would be interesting to extend the study to look at childhood perfusion changes with age. There are large changes in neuronal numbers and density during development which could help to determine if there is a link between neuronal number and perfusion.

- There are many factors other than gender and age that could affect perfusion, for example IQ, brain volume, oestrogen levels, caffeine, artherosclerotic lesions,
blood pressure and dehydration. Investigation into these effects are necessary for clinical studies, where these factors may need to be controlled for.

- Further validation and characterisation of temporal and spatial perfusion correlations are essential. These measurements could have an important contribution to the understanding of perfusion regulation and brain function.

7.4 Applications

Applications in stroke, arteriovenous malformation, motor activation and multiple sclerosis are investigated. Only one stroke patient was scanned but this allowed for a useful comparison between CASL and bolus tracking techniques of perfusion measurement. Reasonable agreement between the two techniques was found. Interesting perfusion measurements were made in arteriovenous malformations, suggesting different vascular behaviour in different brain regions. The findings suggest that CASL could be useful in determining the underlying tissue perfusion while avoiding contamination from the large vessel shunts. The motor activation study showed increased perfusion in the motor cortex of 57% during a finger opposition task. The change in activation signal with delay time helped to validate the two-compartment model. A more extensive study in multiple sclerosis found subtle perfusion changes in the normal appearing white matter of two subgroups of MS patients.

7.4.1 Future work

- In arteriovenous malformations the results are of sufficient interest to warrant an extended study.

- It would be interesting to see if arrival time or permeability changes could be detected during task activation. A study by Gonzalez-At et al. [73] showed reductions in arrival time with activation.

- Further analysis of the MS data by Statistical Parametric Mapping (SPM) may find regional perfusion differences in the grey matter of MS patients compared to normal subjects. Improved image resolution could allow the measurement of lesion perfusion.
• Measurements of PS/vbw in multiple sclerosis could be interesting. An increase in PS/vbw could be indicative of very early damage to the blood brain barrier. Current studies look at changes to gadolinium permeability [199]. Changes to water permeability could perhaps be detected earlier, when the damage to the BBB is too small to change the permeability to gadolinium.

• A study is planned to look at perfusion changes during epileptic seizures. Perfusion is predicted to correlate with changes to electrical activity during a seizure, as recorded by EEG.
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