

Magnetic resonance spectroscopy of the *in vivo* brain with semi-LASER



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This thesis is dedicated
to the memory of my grandma
Gaga

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Abstract

Changes in the metabolic state of the brain can occur, for example, as a result of neuronal activity or in pathologies such as cancer. In these cases, an altered energy demand can lead to changes in neurochemical concentrations detectable using proton magnetic resonance spectroscopy (^1H -MRS). This thesis explores *in vivo* ^1H -MRS methods for detection of such changes in the healthy and diseased brain. Specifically, this thesis aims to develop methods with semi-LASER localisation, thereby minimising the negative effects of chemical shift displacement and field inhomogeneity on spectral acquisition.

Firstly, a Hadamard-encoded semi-LASER method for simultaneous measurement from two regions was developed at 7 T. Slice profiles, with low chemical shift displacement and small amounts of signal overlap, were revealed in phantom and *in vivo*. This was then implemented in a study of neurochemical change during positive and negative blood oxygen level-dependent (BOLD) responses. Negative BOLD responses are thought to reflect regions of neuronal suppression. A small decrease in ascorbate, as well as the T_2^* -induced linebroadening of several spectra, were observed in these regions. Furthermore, increases in glutamate and lactate were detected in positive BOLD regions. These findings suggested that negative BOLD may not be generated by an increase in local GABA concentration.

Secondly, an optimised semi-LASER sequence ($\text{TE} = 110 \text{ ms}$) at 3 T was shown to improve localisation of the oncometabolite 2-hydroxyglutarate (2-HG) – a product of IDH-mutation found in the majority of gliomas. This resulted in improved detection of 2-HG in patients compared to an existing technique. The method was also compared to 7 T, where benefits of an increased spectral resolution resulted in significantly better detection of 2-HG along with associated metabolites.

This thesis highlights the importance of robust localisation for performing sensitive *in vivo* ^1H -MRS neurochemical measurement in the human brain.

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List of Abbreviations

α-KG	α -ketoglutarate
dHb	deoxyhaemoglobin
BOLD	Blood Oxygenation Level-Dependent
CBF	Cerebral Blood Flow
CMRO₂	Cerebral Metabolic Rate of Oxygen
CRLB	Cramér-Rao Lower Bounds
CSF	Cerebrospinal Fluid
FID	Free Induction Decay
FOV	Field Of View
HRMAS	High Resolution Magic Angle Spinning
HLSVD	Hanzel Lanczos Singular Value Decomposition
IDH	Isocitrate Dehydrogenase
MRS	Magnetic Resonance Spectroscopy
NMR	Nuclear Magnetic Resonance
OVS	Outer Volume Saturation
PCA	Principal Component Analysis
PET	Positron Emission Tomography
PRESS	Point REsolved SpectroScopy
PSF	Point Spread Function
RF	Radiofrequency
STEAM	STimulated Echo Acquisition Mode
TCGA	The Cancer Genome Atlas
VOI	Volume Of Interest

List of Metabolites

2-HG	2-hydroxyglutarate
Ala	Alanine
Asc	Ascorbate
Asp	Aspartate
Cr	Creatine
GABA	γ -aminobutyric acid
Glc	Glucose
Gln	Glutamine
Glu	Glutamate
Gly	Glycine
GPC	Glycerylphosphorylcholine
GSH	Glutathione
Ins	<i>myo</i> -inositol
Lac	Lactate
NAA	N-acetylaspartate
NAAG	N-acetylaspartylglutamate
PCho	Phosphocholine
PCr	Phosphocreatine
PE	Phosphorylethanolamine
sIns	<i>scyllo</i> -inositol
Tau	Taurine

Preface

Contributions to the thesis

I would briefly like to draw attention to specific contributions which several people have made to the work presented in this thesis.

Firstly, I acknowledge my supervisor, Dr. Uzay E. Emir, for providing the semi-LASER implementation at 3 T and 7 T which I have modified in this thesis. The scanning of glioma patients was also carried out jointly by myself and Dr. Emir. The optimisation of the semi-LASER echo time (in Section 6.3) was motivated by initial exploratory analysis of the echo times performed by Dr. Emir.

I extend acknowledgement to Dr. Sarah J. Larkin, who conducted the immunological and DNA measurements on glioma samples to determine their IDH-mutation status - the methods of which are summarised in Section 6.6.1. Dr. Larkin also generated the images displayed in Fig. 6.6 of this thesis.

Finally, I also acknowledge the use of the visual stimulation in Chapter 5, which was developed by Dr. André Gouws (for reference see [1]). He also kindly provided me with the *PsychoPy* stimulus code for use in this work.

Publications

The following is a list of publications and conference proceedings which have arisen as a result of the work carried out in this thesis.

Papers

Berrington A, Voets NL, Plaha P, Larkin SJ, Mccullagh J, Stacey R, Yildirim M, Schofield CJ, Jezzard P, Cadoux-Hudson T, Ansorge O, and Emir UE. Improved localization for 2-hydroxyglutarate detection at 3T using long-TE semi-LASER. *Tomography*. 2016;2(2):94-105. doi:10.18383/j.tom.2016.00139

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Ip B, **Berrington A**, Hess AT, Parker AJ, Emir UE, Bridge H. Tracking neurochemical and BOLD-changes in the human visual cortex using simultaneous short echo semi-LASER spectroscopy and BOLD-imaging at 7 Tesla. In: *Society for Neuroscience* San Diego; 2016.

Berrington A, Voets NL, Larkin SJ, de Pennington N, Mccullagh J, Al-Qahtani K, Stacey R, Yildirim M, Jezzard P, Clare S, Schofield C, Ansorge O, Cadoux-Hudson T, Plaha P, Emir UE. Improved detection of 2-hydroxyglutarate with a broad-band semi-LASER sequence at 3 T. In: *ESMRMB*. Edinburgh; 2015

Berrington A, Gouws A, Clare S, Jezzard P, and Emir UE Unravelling the neurochemical mechanism of positive and negative BOLD responses: a combined fMRI-fMRS study. In *ISMRM*. Toronto; 2015

Berrington A, Jezzard P, Clare S, Emir UE. Two-voxel Hadamard encoded semi-LASER spectroscopy for in vivo MRS at ultra-high field. In: *ISMRM*. Milan; 2014

1

Introduction

Motivation : *The Hungry Brain*

The human brain has a big appetite. It is commonly stated that the brain constitutes only 2% of the body's mass, yet consumes around one fifth of the available oxygen (see [2, 3] and references therein). This large energy budget is vital to maintain the many specialised functions of the brain with the majority estimated to be spent on supporting neuronal signalling [4, 5], yet energy is also required to power basic cellular processes [6]. Energy demand in the brain may also change over time or in cases of pathology. For example, changes in the level of neuronal activity may be met by metabolic alterations in neurons and glial cells [7–9] and fluxes through major metabolic pathways can change in response to diseases such as cancer [10–12].

The motivation behind the work presented in this thesis is to develop methods to probe chemical markers of neuronal function and metabolism in the healthy and diseased brain *in vivo*. Such methods have the potential to contribute to a greater understanding of the coupling between neuronal processes and energy metabolism in the living brain and may ultimately lead to improved diagnoses in diseases such as cancer, where concentrations of neurochemical markers are altered. With this in mind, the methods developed in this thesis are applied in two important biological settings. The first such application is a study of metabolic changes during functional imaging signals (BOLD responses). In particular, focus is placed on the detection of neurochemical changes occurring in regions that exhibit *reductions* in neuronal activity (negative BOLD responses). The second application presented in this thesis, is the detection of a metabolic marker of disrupted metabolism in gliomas (2-hydroxyglutarate) and its neurometabolic signature, with the aim to improve *in vivo* diagnosis.

Many measurement techniques are available to answer such questions, yet proton magnetic resonance spectroscopy (^1H -MRS) presents itself as a clear and versatile tool for use in the living human brain. Positron emission tomography (PET) studies, for example, can provide detail on local glucose consumption [13], yet information on levels of neurotransmitter or other key metabolites is lacking. MRS measurements using other nuclei, such as ^{13}C , can offer unprecedented measurement of dynamic changes in metabolism [14, 15]. However, due to its low natural abundance *in vivo*, suffers from low sensitivity. The availability of hardware used for MR imaging (MRI), and the higher intrinsic signal yield from proton, places ^1H -MRS in a unique position for measurement of the metabolic state of the brain [16, 17]. In addition, advances in MR technology, such as the advent of ultra-high field ($\geq 7\text{ T}$) imaging systems, present ^1H -MRS with the potential for significantly improved sensitivity to detect neurochemical changes. However, several challenges of localising signal *in vivo* with ^1H -MRS, such as the spatial displacement between individual chemical resonances (chemical shift displacement) are present and such problems, along with a large magnetic field inhomogeneity, can be made considerably worse at ultra-high field.

The semi-LASER (localisation by adiabatic selective refocusing) method is a promising technique for MRS in the brain [18, 19]. This method relies on the use of broadband adiabatic pulses, which are robust to some of the challenges that face *in vivo* MRS (such as chemical shift and B_1 inhomogeneity) [20]. As a result, the sensitivity of MRS methods to measure small changes in the metabolic state of the brain may benefit considerably from this technique. The semi-LASER method for MRS is, therefore, used extensively in this thesis, and the importance of considering robust localisation of the MRS signal on the spectral profile is highlighted in the various applications of this thesis for study of *in vivo* metabolism in the brain.

Thesis Outline

This thesis will begin with a short description of the underlying physical principles surrounding ^1H -MRS, including the use of adiabatic pulses and the semi-LASER localisation scheme in Chapter 2.

A description of the biological questions of brain metabolism that are targeted in this thesis is given in Chapter 3. In the first half of this chapter, an overview of the origin of BOLD responses in the context of metabolic demand, haemodynamics and neurochemical changes is presented. In particular, the current understanding surround negative BOLD responses and their connection with decreases in neuronal activity is reviewed. The metabolism of neuronal inhibition, its connection to blood flow changes and the role of γ -aminobutyric acid (GABA) are also discussed. In the second half of this chapter, the metabolic impairment arising from the acquisition of IDH-mutation in glioma is described. In particular, the role and detection of 2-hydroxyglutarate is discussed.

In Chapter 4 an acquisition method for simultaneous MRS measurement from two regions at 7 T is presented. A semi-LASER sequence is modified with Hadamard encoded excitation pulses, and tested in phantoms and then *in vivo* in five healthy volunteers. This same two-voxel method is then applied in the study of neurochemical changes during negative and positive BOLD responses. Chapter 5 thus reports the results of a novel functional MRS (fMRS) study of BOLD responses. In particular, the hypothesis of whether negative BOLD regions reflect regions of inhibition and lower metabolic demand is examined.

Methodological development of a semi-LASER sequence is then presented at 3 T in Chapter 6 in an attempt to improve the *in vivo* detection of the metabolite 2-hydroxyglutarate, specific to IDH-mutation in glioma cells. The modified sequence is compared to an existing MRS method using simulations, phantoms and ultimately studies on eleven glioma patients.

The optimised method (at 3 T) is then compared against a similar existing semi-LASER sequence at 7 T in Chapter 7. Through comparative work in phantoms and patients the detectability of 2-hydroxyglutarate is compared at both field strengths. In addition, the sensitivity of semi-LASER to detect the metabolic signature of individual mutations is examined.

Finally, a general conclusion of the findings presented in this thesis can be found in Chapter 8, together with suggestions for future research directions.

Magnetic Resonance Spectroscopy: Technical Background

A brief history of NMR

In 1983, Behar *et al.* demonstrated the use of proton nuclear magnetic resonance (NMR) in rat brain, marking the birth of *in vivo* proton magnetic resonance spectroscopy (^1H -MRS) of the brain [21]. The origins of NMR began years earlier in 1939 with the discovery by Rabi *et al.* that application of oscillating magnetic fields can generate detectable transitions in nuclear spin states [22]. In 1946, two further studies discovered that irradiation of nuclear spins, in solids and liquids placed in a magnetic field, resulted in absorption and subsequent emission of radiation at a resonant frequency [23, 24]. The importance of these discoveries was recognised through the award of a *Nobel prize in physics* in 1944 for Rabi and joint prizes in 1952 for Purcell and Bloch for work on the measurements of nuclear magnetic moments [25]. NMR rapidly evolved as a measurement technique in physics but also crucially as a method for probing biological samples *in vivo*. In particular, early studies were carried out using ^1H and phosphorus nuclei ^{31}P in biological samples [26, 27].

^1H -MRS has since evolved to become an incredibly useful modality for *in vivo* neurochemical measurement in the brain [28], capable of quantification of around 15 different neurochemicals at 7 T including, N-acetylaspartate (NAA), choline (Cho), creatine (Cr), inositol (Ins), glutamate (Glu), glutamine (Gln) and γ -aminobutyric acid (GABA) [29]. Considerable work has also been carried out on many other MR-visible nuclei in studies on neurotransmitter cycling [15, 30] (^{13}C), oxygen metabolism [31] (^{17}O) and phospholipids [32] (^{31}P), for example. The basic principles of NMR ultimately led to the development of magnetic resonance imaging (MRI) in 1973 [33, 34], for which Lauterbur and Mansfield later received the *Nobel prize in medicine or physiology* in 2003.

This chapter offers a brief general description of NMR and by extension MRS. Several useful articles and book chapters were consulted in preparation for this section. The interested reader is referred to these for greater detail [35–38].

NMR is concerned with transitions of individual nuclear spin states under irradiation, thus a quantum mechanical approach is required to fully explain the principles. In particular, it is needed to understand the nature of coupled spins and spin evolution. Later in this chapter, a brief overview of the classical picture of NMR is presented, which provides insight for localisation and adiabatic pulses.

2.1 Quantum Mechanical Description

The quantum mechanical description of NMR is not concerned with net magnetisation, but rather transitions between energy states of a nucleus. An external magnetic field, B_0 , interacts with degenerate nuclear spin states, splitting them and allowing transitions between individual energy levels within the nucleus – the Zeeman effect. NMR, and by extension MRS, are thus concerned with the detection of these transitions between the nuclear spin energy states, observable using radiation in the radiofrequency spectrum.

The interested reader is referred to several useful texts which have been consulted for topics in this section [38–40]. A recap of quantum mechanical notation, including operators formalism in NMR, is given by Levitt (2008) [38]. This section focusses on the proton only, since this thesis is concerned with ^1H -MRS. The proton possesses a nuclear spin angular momentum, $I = \frac{1}{2}$.

2.1.1 Single spin- $\frac{1}{2}$

Analogous to a current dipole in classical physics, the nuclear spin possesses a magnetic moment, $\hat{\mu}$ which is fundamentally linked to its angular momentum $\hat{I} = (\hat{I}_x, \hat{I}_y, \hat{I}_z)$,

$$\hat{\mu} = \gamma \hat{I}, \quad (2.1)$$

where γ is the gyromagnetic ratio for the nucleus (for protons $\gamma = 42.6$ MHz/T). If the spin is placed in an external magnetic field $\vec{B}$ the magnetic energy for such a system is given by the Hamiltonian, $\hat{H}_{\text{mag}}$, such that

$$\hat{H}_{\text{mag}} = -\hat{\mu} \cdot \vec{B}. \quad (2.2)$$

Applying applying an external field along the positive z-direction only, $\vec{B} = (0, 0, B_0)$, Eqn. 2.2 becomes

$$\hat{H}_{\text{mag}} = -\gamma B_0 \hat{I}_z. \quad (2.3)$$

For a spin- $\frac{1}{2}$ nucleus, two spin states exist, namely $|\alpha\rangle = \begin{pmatrix} 1 \\ 0 \end{pmatrix}$ and $|\beta\rangle = \begin{pmatrix} 0 \\ 1 \end{pmatrix}$, which represent the longitudinal component of the spin angular momentum aligned and anti-aligned with the field along z. These states are also known as spin-up and spin-down states and are eigenvectors of the longitudinal angular momentum operator $\hat{I}_z$, such that

$$\hat{I}_z |\alpha\rangle = +\frac{\hbar}{2} |\alpha\rangle \quad \text{and} \quad \hat{I}_z |\beta\rangle = -\frac{\hbar}{2} |\beta\rangle, \quad (2.4)$$

with corresponding energies (Eqn. 2.3) given by

$$\hat{H}_{\text{mag}} |\alpha\rangle = -\frac{\hbar}{2} \gamma B_0 |\alpha\rangle \quad \text{and} \quad \hat{H}_{\text{mag}} |\beta\rangle = +\frac{\hbar}{2} \gamma B_0 |\beta\rangle. \quad (2.5)$$

Thus, the spin-up state $|\alpha\rangle$ is of lower energy than spin-down state $|\beta\rangle$, since it is aligned with the external magnetic field. The energy difference, ΔE , between these two states is then

$$\Delta E = \hbar \gamma B_0. \quad (2.6)$$

Fig. 2.1 shows the energy level diagram for this system.

Transition between these two energy states can occur with the absorption or emission of photon of energy ΔE . The energy of the photon is quantised in units of $\hbar$ such that, $E = \hbar \omega$, where ω is the angular frequency. From (Eq. 2.6), this results in the Larmor condition

$$\omega = \gamma B_0. \quad (2.7)$$

Absorption of a photon of frequency equal to the the Larmor frequency generates a transition in NMR.

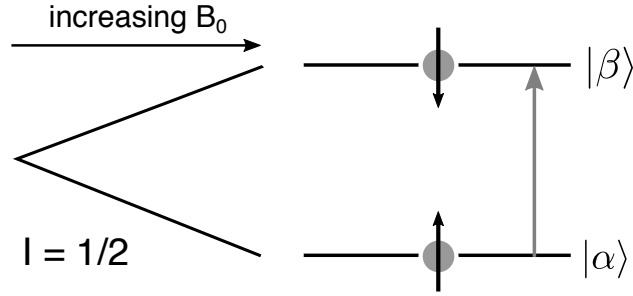


Figure 2.1: The effect of an external magnetic field, B_0 , on a single spin- $\frac{1}{2}$ nucleus. The field splits the energy levels associated with the aligned and anti-aligned longitudinal angular momentum states, $|\alpha\rangle$, and $|\beta\rangle$, respectively.

2.1.2 Two spin- $\frac{1}{2}$ system

For two spin- $\frac{1}{2}$ nuclei, there are $2^2 = 4$ possible eigenstates, corresponding to every combination of spin-up and spin-down for both nuclei. Such a basis can be fully described by $|\alpha\alpha\rangle$, $|\alpha\beta\rangle$, $|\beta\alpha\rangle$ and $|\beta\beta\rangle$. The energy level diagram for this system is shown in Fig. 2.2 (left). If the two spins are non-interacting there will be two measured frequencies corresponding to two unique transitions within the system.

2.1.3 J-coupling

A system containing two uncoupled spins (I , S) results in four states with two unique transition energies (see Fig. 2.2 [left]). In this setting only two spectral lines are observed, since a transition of I described by $|\alpha\rangle \rightarrow |\beta\rangle$ requires the same energy whether S is in a $|\beta\rangle$ or $|\alpha\rangle$ state.

J-coupling is an interaction between I and S mediated by the electrons. Information on the magnetisation state of spin S is transferred to spin I via the chemical bond [36, 41]. The strength of this coupling, J_{IS} , is independent of the external field strength and can be negative or positive. The Hamiltonian for this coupling can be expressed as

$$\hat{H}_J = 2\pi J_{12} \hat{I}_I \cdot \hat{I}_S. \quad (2.8)$$

Negative values of the coupling constant, thus, result in a lower energy state when both coupled spins are parallel and higher when anti-parallel. Fig. 2.2 (right) shows the effect of J-coupling and the available energy transitions. J-coupling has two main

consequences. The first is splitting of these energy levels and hence spectral peaks, with a width equal to J , and the second is a modulation of the spin evolution over time.

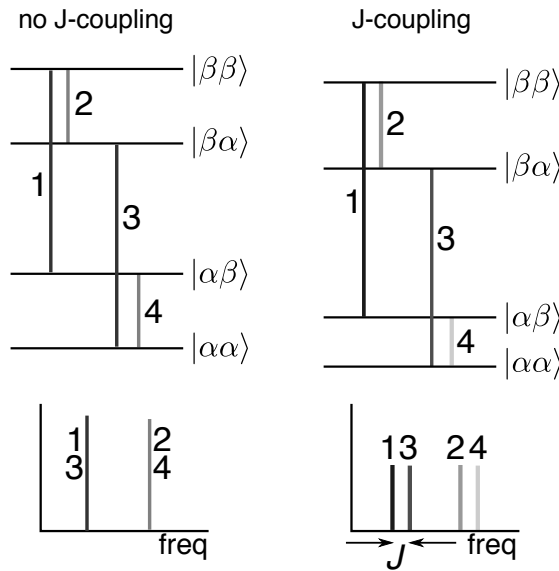


Figure 2.2: For a system of two spins there are two unique transition frequencies (left). However, if coupling is present (right), then the energies associated with individual spin transitions are further split depending on the state of the second spin. This coupling results in a splitting of the individual transitions with a frequency separation, J .

2.1.4 Density Matrix Approach

In order to measure the evolution of any spin system over time, including during interaction with the external field, RF pulses and the effect of J-coupling, density matrix formalism can be used. From a quantum mechanical perspective, the state of a system is fully described by its wavefunction, ψ . During an NMR experiment, the evolution of ψ over time, t , such that $\psi(t=0) \rightarrow \psi(t=T)$, is of primary interest.

Any wavefunction can be expressed as a linear combination over a particular basis set $|\phi_n\rangle$ with probability amplitudes, c_n , such that,

$$\psi = \sum_{n=1}^N c_n |\phi_n\rangle. \quad (2.9)$$

In order to measure the state of a system as a function of time, the density matrix approach is a useful tool. Returning to the case of a single spin- $\frac{1}{2}$ nucleus, the state

of the system can be expressed as a superposition over spin-up and spin-down states, $|\alpha\rangle$ and $|\beta\rangle$ (see Eq. 2.9) such that,

$$\psi = c_\alpha |\alpha\rangle + c_\beta |\beta\rangle, \quad (2.10)$$

where c_α^2 and c_β^2 are the probabilities of observing the spin in state $|\alpha\rangle$ and $|\beta\rangle$, respectively. The density matrix operator $\hat{\sigma}$ is defined such that, $\hat{\sigma} = |\psi\rangle \langle\psi|$, or

$$\hat{\sigma} = \begin{pmatrix} c_\alpha c_\alpha^* & c_\alpha c_\beta^* \\ c_\beta c_\alpha^* & c_\beta c_\beta^* \end{pmatrix}. \quad (2.11)$$

The density matrix is a useful construct, since it contains information about each of the possible states of a system. Strictly, each term in the density matrix operator, e.g. $c_\alpha c_\beta^*$, are averages over an ensemble of spins in an NMR sample.

The evolution of the spin system in NMR can be described with $\hat{\sigma}$. In particular, the evolution of this operator over time $\hat{\sigma}(t)$ is described by the Liouville-von Neumann equation [39]

$$\frac{d\hat{\sigma}}{dt} = -i[\hat{H}(t), \hat{\sigma}(t)], \quad (2.12)$$

for Hamiltonian $\hat{H}$, which can contain terms describing chemical shift evolution, J-coupling and time-dependent RF. The solution of Eq. 2.12 at a time t_k can be shown to be,

$$\hat{\sigma}(t_k) = e^{-i\hat{H}_k t_k} \hat{\sigma}(0) e^{i\hat{H}_k t_k}. \quad (2.13)$$

Thus, evolution of the density matrix can be calculated over successive time intervals, t_k , and determined using the propagators, $e^{-i\hat{H}_k t_k}$.

J-evolution

The density matrix can be expressed in a basis of the angular momentum operators, each of which represent an orthogonal component of magnetisation. Using the density matrix formalism, it can be shown that, for a coupled spin-system (I, S) that evolves

over time under the influence of J-coupling (Eqn. 2.8), a spin that is initially tipped into the transverse plane ($\sigma(0) = I_y$) will evolve such that at a time, τ , the density becomes,

$$\sigma(\tau) = I_y \cos(\pi J_{IS}\tau) - 2I_x S_z \sin(\pi J_{IS}\tau), \quad (2.14)$$

where the weakly coupled spin approximation has been used ($|\delta_1 - \delta_2| \gg J_{IS}$) [39]. For strongly coupled spins the evolution of the spin system is much harder to predict. Here the density matrix has acquired a time dependent phase shift on the y -component of the spin I . In addition, the component $2I_x S_z$ appears, which is termed ‘anti-phase’ coherence between the spin I and S . Such a component represents magnetisation of spin I , which is oppositely phased compared to the coupling with S . The modulation occurs with a frequency J (in Hz). At a time $\tau = \frac{1}{2J}$, the state will be a purely anti-phase coherence state, whereas at a time $\tau = \frac{1}{J}$ the state will be inverted compared to its initial state. The result of this is a modulation, such that individual J-coupled resonances in the spectrum acquire a phase as a function of time.

2.2 Classical Description

The classical description of Eq. 2.7 is that of a rotating magnetisation vector, $\vec{M}$. Very briefly, the resultant magnetisation $\vec{M}$ of a sample per unit volume is given by the sum over the individual magnetic moments

$$\vec{M} = \sum_n \vec{\mu}. \quad (2.15)$$

Given each individual magnetic moment will tend to align or anti-align with the external field, there will be a net magnetisation along the z -axis. On average the transverse component of the magnetic moments will be distributed randomly in the transverse plane (xy), thus cancelling to zero. Considering the populations of spins in $|\alpha\rangle$ and $|\beta\rangle$ states it can be shown that the net longitudinal magnetisation, M_0 , from n spins at thermal equilibrium is given by

$$M_0 = (\gamma\hbar)^2 \frac{nB_0}{4kT}, \quad (2.16)$$

where k is the Boltzmann constant and T is the temperature of the sample.

2.2.1 Bloch Equations

Bloch demonstrated the time evolution of the net magnetisation in the presence of an external magnetic field [42, 43] can be described by,

$$\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B} - \frac{1}{T_1} \begin{pmatrix} 0 \\ 0 \\ M_z - M_0 \end{pmatrix} + \frac{1}{T_2} \begin{pmatrix} M_x \\ M_y \\ 0 \end{pmatrix}, \quad (2.17)$$

where T_1 and T_2 are the characteristic decay times for longitudinal and transverse components of magnetisation to return to its equilibrium value, respectively. Neglecting this relaxation of the signal (T_1 and $T_2 \rightarrow \infty$) then the solution can be written

$$M_{xy}(t) = M_{xy}(0) e^{-i\gamma B_0 t}, \quad (2.18)$$

$$M_z(t) = M_0. \quad (2.19)$$

where the $\vec{B}$ is applied along the z -direction and $M_{xy} = M_x + iM_y$. Eqn. 2.18 thus describes circular motion of the magnetisation about z in the xy plane with a frequency, $\omega = \gamma B_0$. This is identical to the Larmor condition of Eqn. 2.7.

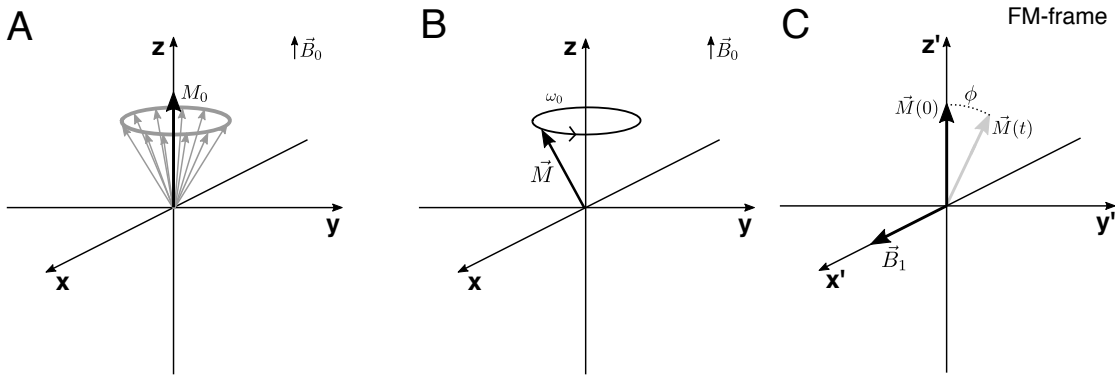


Figure 2.3: **A:** Sum over all magnetic moments results in a net magnetisation along the z -axis. **B:** If given an initial transverse component, spins precesses at $\omega_0 = \gamma B_0$ about the applied field. **C:** In a frame which rotates at ω_0 , $\vec{M}$ appears stationary. If an RF pulse is applied then $\vec{M}$ will precess around the direction of $\vec{B}_1$

Eqn. 2.18 implies that if magnetisation has an initial transverse component, i.e. $M_{xy} \neq 0$, it will precess around the external field with a frequency equal to the Larmor frequency. It is this precession that is observable in MRS, through a combination of excitation of magnetisation into the transverse plane and detection of the resultant current induced in quadrature coils positioned along x and y dimensions.

2.2.2 RF Nutation

The Bloch equation (Eqn. 2.17) also provides a useful tool for visualising the effect of a radiofrequency (RF) pulse of duration, T , with magnetic field, $\vec{B}_1$, on the magnetisation. For this purpose it is useful to transform to a coordinate system which rotates at the Larmor frequency (see Fig. 2.3C). In this frame, $\vec{M}$ remains stationary until acted upon by the applied field $\vec{B}_1$. $\vec{M}$ will then precess around the applied field B_1 , analogous to the case of the applied external field, B_0 (Eqn. 2.17). The angle, ϕ , through which the magnetisation precesses is the flip-angle of the pulse and is determined by the amplitude and duration of the RF pulse, such that,

$$\phi = \int \gamma B_1 dt \quad (2.20)$$

2.2.3 Relaxation

If relaxation of the magnetisation occurs either longitudinally by ‘spin-lattice’ or T_1 -decay, or transversally via ‘spin-spin relaxation’ or T_2 -decay, the solutions of the Bloch equations (2.17), in the absence of a B_1 -field and calculated in the rotating frame, become,

$$M_{xy}(t) = M_{xy}(0)e^{-t/T_2}, \quad (2.21)$$

and equally for the longitudinal component, $M_z(t)$,

$$M_z(t) = M_0(1 - e^{-t/T_1}), \quad (2.22)$$

where the initial longitudinal component of magnetisation has been assumed to be zero.

The exponential transverse decay, T_2 , is particularly problematic for MRS using long-TE sequences or ultra-high field acquisition where T_2 values shorten. In practice T_2 -decay actually happens with a rate T_2^* , which accounts for dephasing due to magnetic inhomogeneities in the sample.

2.3 Localisation

2.3.1 Chemical Shift

The chemical environment of a nuclear spin affects its resonant frequency [44]. It is this basic principle that forms the basis of an NMR/MRS spectrum. Electrons within a molecule will generate their own magnetic moment, which opposes the applied field, centred on the nucleus. The result of this ‘electron shielding’ is that the static magnetic field, B_0 , experienced by the nucleus, is reduced by a dimensionless factor σ , such that

$$B = B_0(1 - \sigma), \quad (2.23)$$

or equivalently, in terms of the resonant frequency,

$$\omega = \omega_0(1 - \sigma). \quad (2.24)$$

Each spin has its own resonant frequency, ω , depending on the chemical environment. The more shielded the spin is, the lower the Larmor frequency for this spin. Eqn. 2.24 also implies that the amount of frequency shift is dependent on the magnitude of the applied field. Thus, a field-independent measure of the chemical shift is defined, δ , relative to a standard frequency such that

$$\delta = \frac{\omega - \omega_{\text{ref}}}{\omega_{\text{ref}}} \cdot 10^6 \quad (2.25)$$

measured in parts per million [ppm].

2.3.2 Frequency-based Localisation

In vivo MRS, as opposed to NMR, requires measurement from a localised volume within a sample. This can be achieved through the use of field gradients. Ordidge *et al.* first described a technique called image-selected *in vivo* spectroscopy (ISIS), which performed 3D localisation with gradients, yet required eight acquisitions (or shots) to achieve this [45]. The majority of popular localisation techniques in ^1H -MRS, make use of single-shot techniques, and thus they form the basis of the following section.

A spatial magnetic field gradient, $\vec{G} = (G_x, G_y, G_z)$, experienced at position $\vec{r} = (x, y, z)$ will generate an additional magnetic field $\vec{G} \cdot \vec{r}$. Equivalently, across one dimension this results in a change to the Larmor frequency of

$$\omega = \omega_0 + \gamma G_x x. \quad (2.26)$$

A graphical representation of Eqn. 2.26 in the rotating frame is shown in Fig. 2.4. Suppose an RF pulse, of fixed bandwidth, $\Delta\omega$, is played out at the same time as the gradient, G_x . The pulse will only be experienced by spins that lie within $\Delta\omega$, thus satisfying the Larmor frequency in Eqn. 2.26.

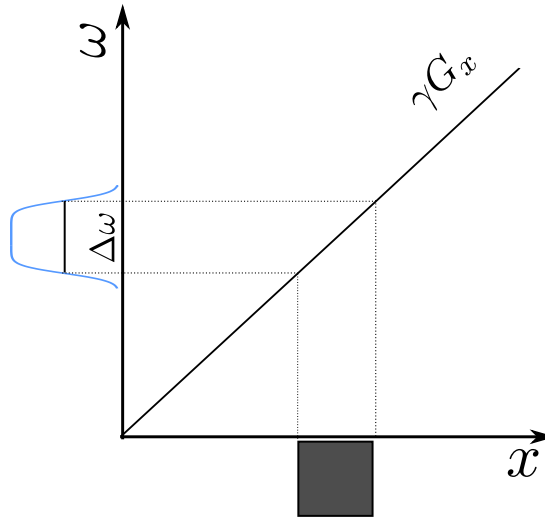


Figure 2.4: Application of a gradient during an RF pulse will result in selection of spins which satisfy the Larmor condition within the frequency band $\Delta\omega$.

However, Eqn. 2.26 neglects the different chemical shift of each nucleus.

2.3.3 Chemical Shift Displacement

All single-shot sequences, which use gradient localisation with frequency-selective pulses, suffer from chemical shift displacement. Since a spin, A , will have a resonant frequency, ω_A , and similarly spin B will resonate at ω_B (Eqn. 2.24), there will be a difference in spatial localisation (Eqn. 2.26) between the two spins Δx according to,

$$\Delta x = \frac{\omega_A - \omega_B}{\gamma G_x}. \quad (2.27)$$

Since the voxel size, V_x , generated by a pulse of spectral bandwidth $\Delta\omega$, is given by,

$$V_x = \frac{\Delta\omega}{\gamma G_x}, \quad (2.28)$$

we can rewrite Equation (2.27) as,

$$\Delta x = \frac{\omega_A - \omega_B}{\Delta\omega} V_x. \quad (2.29)$$

The effect of Eqn. 2.29 on localisation is plotted in Fig. 2.5.

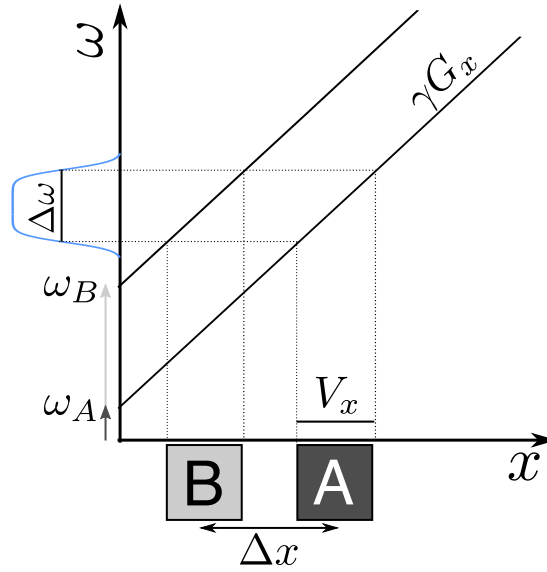


Figure 2.5: Localisation of two spins with different chemical shift values ω_A and ω_B . The difference in shift can lead to a spatial displacement between the voxel locations for these two spins.

Equation (2.29) implies that:

1. Spins with a greater difference in frequency will be shifted further apart, which means chemical shift displacement increases with field strength.
2. Higher bandwidth pulses, or equivalently stronger gradients, are desirable to minimise this chemical shift artefact for constant voxel sizes.

When localising in three dimensions, chemical shift displacement occurs along x , y and z , and can result in only a small degree of overlap between voxels across metabolites. This is a particular issue for coupled spin systems, where different parts of

the same spin system experience different pulses depending on their relative chemical shift and can lead to compartmental evolution of signal within a voxel – known as the chemical shift displacement artefact [46].

2.4 Ultra-high Magnetic Fields

MRS can potentially benefit from acquisition at ultra-high magnetic field (UHF) (≥ 7 T). Assuming sample losses dominate the noise signal and the effect of RF penetration into the sample is negligible, then the signal-to-noise ratio (SNR) should increase roughly linearly with the magnetic field B_0 [47–49],

$$\text{SNR} \propto B_0. \quad (2.30)$$

In addition, for MRS there is a significant benefit of an increased chemical shift dispersion (Eq. 2.24) and, hence, greater spectral resolution, which increases linearly with the applied field. However, these gains come at the price of a greatly enhanced chemical shift displacement (Eqn. 2.29).

In addition, metabolite linewidths ($\nu \sim 1/T_2$) increase due to shorter T_2 -decay times and increased susceptibility gradients at high fields [50]. The larger susceptibility gradients at UHF also result in a greater inhomogeneity in the B_0 distribution across the brain [51, 52]. SNR improvements at higher field strengths can, therefore, fall below the theoretical limit [53], unless adequate localised shimming is achieved [54, 55].

Acquisition is also made difficult by large inhomogeneities in the B_1 field, arising from interaction and interference of the B_1 field within the sample itself [56, 57]. In the brain, this can often lead to increased signal in the centre with a peripheral decrease in available signal. Acquisition using hardware such as parallel transmit RF arrays (for review see: [58]) or the use of dielectric pads [59] can mitigate against the loss of B_1 within regions. An alternative approach is to use adiabatic pulses to perform frequency selective localisation.

2.5 Adiabatic Pulses

Adiabatic RF pulses are ideally suited to achieve a uniform RF nutation across a sample in regions of B_1 inhomogeneity. In addition, due to their broadband frequency content, adiabatic pulses are particularly good for improving chemical shift displacement. Adiabaticity is achieved by performing a ‘frequency sweep’ of the sample in question. Initially in NMR, adiabatic conditions were achieved by sweeping the frequency of the main magnetic field, B_0 , under continuous wave RF irradiation [20]. However, current techniques with static B_0 fields, modulate the carrier frequency (or equivalently phase) of the RF pulse itself. Excellent reviews of the concepts underlying generation of the adiabatic condition are given by Tannús and Garwood [60] and later by Garwood and DelaBarre [20]. Here a brief overview of adiabaticity is given using the classical picture of NMR.

2.5.1 Effective Magnetisation

Consider the rotating frame of reference ($x' y' z'$), which rotates at the instantaneous frequency of the RF pulse, ω_{RF} , around z' . In this frequency-modulated frame, the direction of the RF field, $\vec{B}_1$, is fixed (chosen to be along x'). If the RF frequency is identical to the Larmor frequency, $\omega_{RF} = \omega_0$, the magnetisation will simply precess around $\vec{B}_1$ (Fig.2.3C).

Suppose that the frequency of the pulse is modulated over time, $\omega_{RF}(t)$. If $\omega_{RF}(t) \neq \omega_0$, an off resonance component of the field will be generated along z' such that $\Delta\omega = \omega_0 - \omega_{RF}(t)$ (Fig. 2.6 A). The resultant component of the two fields, B_1 and $\Delta\omega/\gamma$, generates an effective magnetic field $\vec{B}_e$.

Over the course of the pulse, ω_{RF} starts below ω_0 , such that $\omega_{RF}(t=0) \ll \omega_0$ and ends with $\omega_{RF} \gg \omega_0$, with $\vec{B}_e$ thus sweeping an angle α about y' over the course of the pulse (Fig. 2.6), such that

$$\alpha(t) = \tan^{-1} \left(\frac{\gamma B_1(t)}{\Delta\omega(t)} \right). \quad (2.31)$$

To picture the motion of the magnetisation vector $\vec{M}$ during this pulse, it is useful to once again transform coordinates into a frame where the effective magnetisation,

$\vec{B}_e$ is fixed along z'' . This frame ($x'' y'' z''$) thus rotates about y' at the instantaneous rate of change of the effective field, $\frac{d\alpha(t)}{dt}$ (Fig. 2.6B). Rotation of $\vec{B}_e$ about y' thus generates a new field in this frame along y'' , of magnitude $\gamma^{-1} \frac{d\alpha(t)}{dt}$.

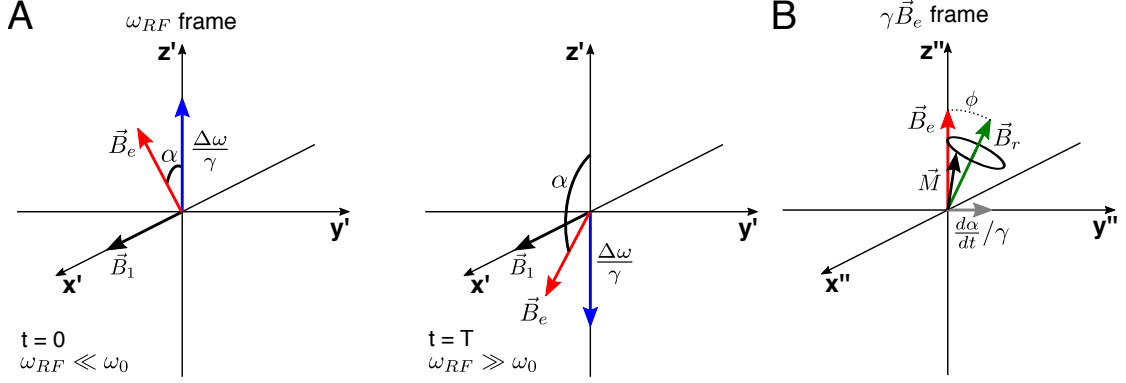


Figure 2.6: Picturing the adiabatic condition using rotating frames. **A:** In a frame which rotates with frequency of the RF pulse ω_{RF} , a frequency-swept pulse will generate an effective field B_e with components from B_1 and $\Delta\omega/\gamma$ when ω_{RF} deviates from the Larmor frequency, ω_0 . **B:** Transformation to a frame rotating with B_e reveals a resultant field B_r dependent on the rate of change of the effective field $\frac{d\alpha(t)}{dt}$. Magnetisation will follow the effective field provided this rate of change is small.

2.5.2 Adiabatic Condition

A new resultant magnetic field, $\vec{B}_r$ is thus generated from $\vec{B}_e$ and $\frac{d\alpha(t)}{dt}$, around which the magnetisation, $\vec{M}$, will precess. However, provided that the rate of change of effective field is small compared to its magnitude, the magnetisation will remain closely aligned with $\vec{B}_e$. This is the adiabatic condition, namely,

$$|\gamma \vec{B}_e| \gg \left| \frac{d\alpha(t)}{dt} \right|. \quad (2.32)$$

Provided this condition is satisfied, $\vec{M}$ will follow $\vec{B}_e$ through the duration of the pulse. The design of adiabatic pulses is therefore concerned with choosing appropriate frequency, $\Delta\omega(t)$, and amplitude, $\omega_1(t) = \gamma B_1(t)$, modulations of the pulse to satisfy this general condition.

For cases when the frequency sweep ends ($t = T$) at the Larmor frequency $\Delta\omega(T) = 0$ or greater than the radiofrequency field $\Delta\omega(T) \gg B_1(T)$, the orientation of the effective field, $\vec{B}_e$, is independent of $\vec{B}_1$ field strength. Thus, adiabatic pulses which

perform 90° flips called adiabatic half passage (AHP), and 180° inversions called adiabatic full passage (AFP), are largely insensitive of the B_1 -amplitude.

It is noted that the above is only applicable to on-resonance spins. During spatial localisation with gradient fields, spins possess slightly different Larmor frequencies, thus will reach resonance at different times during the frequency sweep. The path traced out by such magnetisation vectors will align with an effective field far from the ideal case, resulting in poor inversion, particularly at either edge of the frequency band [20]. In addition, spins at different spatial positions will accrue differential phase following adiabatic inversion dependent on their $\vec{B}_e$ trajectory, and hence B_1 amplitude, thus resulting in a non-linear phase across the spectrum [61]. For this reason, Connolly *et al.* suggested playing out an identical pulse following the inversion pulse, which can fully reverse this phase accrual [62].

A commonly used adiabatic modulation for AFP inversion is the hyperbolic secant (HS), originally described in [63, 64]. The advantage of this pulse is that the specific modulation of amplitude $\omega_1(t)$ provides a more uniform inversion for all resonances across the inversion band, due to the effective magnetisation aligning parallel with z' for all frequency offsets [20]. Tannus and Garwood have modified HS pulses to generate a flattened pulse with lower RF peak power, called HSn pulses [65]. Further details can be found in Appendix B.

2.6 MRS sequences

Spin Echo

The spin-echo is a pulse-sequence capable of refocussing lost signal from local magnetic field inhomogeneity, T_2^* . The spin-echo can be generated by an initial excitation with a 90° pulse followed by inversion after time t with a 180° [41, 66]. Once in the transverse plane, spin de-phasing according to T_2^* -relaxation occurs resulting in a loss of transverse coherence. Spins are then inverted by the application of the 180° pulse, which reverses the loss of coherence due to inhomogeneities. At the echo time ($TE = 2\tau$) the signal reaches a maximum and T_2^* has been refocussed. However,

spins still decay according to T_2 relaxation. In addition, such a sequence is unable to refocus the evolution due to J -coupling.

2.6.1 PRESS

One of the most common localisation methods *in vivo* is the point resolved spectroscopy (PRESS) sequence [67, 68], which uses three RF pulses for localisation. The basic scheme is $90^\circ - \tau_1 - 180^\circ - \tau_2 - 180^\circ - \tau_3$, with the final inversion pulse occurring on the first echo. Refocussing of the echo, neglecting T_2 relaxation and J -modulation, will occur when $\tau_1 + \tau_3 = \tau_2$. The sequence, therefore, generates a spin echo at $TE = 2\tau_2$. The PRESS sequence has advantages over alternative localisation schemes, such as stimulated echo acquisition mode (STEAM), in that it is able refocus all the available signal [69, 70]. The significant disadvantage of the PRESS sequence is the use of conventional refocussing pulses with limited available bandwidth, and hence large chemical shift displacement.

2.6.2 LASER and semi-LASER

Garwood *et al.* proposed an MRS sequence that makes use of adiabatic refocussing to reduce both the susceptibility to B_1 inhomogeneity at 4 T and also the chemical shift displacement [20]. This sequence, called localisation by adiabatic selective refocussing (LASER), comprises of an AHP excitation followed by three pairs of frequency selective AFP pulses, which refocus magnetisation along all three spatial dimensions. While this sequence offers excellent slice selection profiles in all three dimensions and reduced chemical shift [71], the minimum TE available is long owing to the use of six adiabatic inversion pulses. LASER is similar to the Carr-Purcell (CP) sequence, which makes use of multiple refocussing with 180° pulses following excitation [72]. Such a sequence has the advantage of reduced apparent T_2 decay, owing to minimised diffusion which occurs during interpulse delays, τ_{cp} [73]. Furthermore, it has been shown that modulation due to J -coupling is significantly reduced using a CP-sequence [74]. In particular, for a coupled spin separated by chemical shift δ and having coupling

constant J then provided,

$$\tau_{cp} \sqrt{\delta^2 + J^2} < 1, \quad (2.33)$$

the J-evolution of the spin system is suppressed [36, 74, 75]. For strongly coupled spin systems, CP-sequences can, therefore, be of significant benefit for detection.

At higher field strengths, where RF power requirements are increased, available power is limited. Thus, pulses require longer duration in order to achieve the same flip angle. Longer duration pulses limit the minimum echo time (TE) available to the sequence, yet short-TE sequences are preferable for acquisition of J -coupled resonances. At higher fields, T_2 -values also decrease. With consideration of these problems, Öz and Tkáč modified a LASER sequence by replacing the AHP pulse and first AFP pulse-pair with a short duration non-adiabatic excitation [18]. This sequence, called semi-LASER, allows much shorter TEs than LASER. When compared with a TE = 24 ms on their 4 T system, the sequence produced near identical spectra to a STEAM acquisition at much shorter TE = 5 ms. The semi-LASER scheme is shown in Fig. 2.7; it is defined by the interpulse durations, τ_1 , τ_2 , τ_3 , τ_4 and τ_5 . Localisation is carried out using five selective inversion pulses described by 90° - τ_1 - 180° - τ_2 - 180° - τ_3 - 180° - τ_4 - 180° - τ_5 . A spin echo is completely refocussed provided that $\tau_1 + \tau_3 + \tau_5 = \tau_2 + \tau_4$, thus yielding an echo at TE = $2(\tau_2 + \tau_4)$.

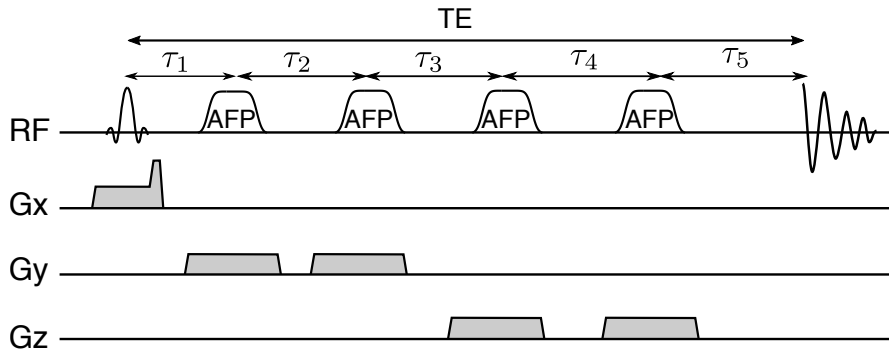


Figure 2.7: The semi-LASER acquisition scheme, with interpulse delays of τ_1 , τ_2 , τ_3 , τ_4 and τ_5 . Pairs of adiabatic full passage (AFP) pulses invert magnetisation along two spatial dimensions, whereas a standard excitation pulse is carried out using the first dimension.

The removal of one pair of AFP pulses sacrifices some of the chemical shift displacement advantages in the excitation direction, however it has still been shown to

perform much better than similar PRESS sequences [76].

2.7 Multi-voxel methods

2.7.1 MRSI

By adding phase encoding gradients to the localisation sequence, akin to MRI, spectroscopic information can be encoded across a 2D or 3D spatial grid [77]; this is often called chemical shift imaging (CSI) or MRS imaging (MRSI). In the brain, MRSI is particularly useful clinically for tumour delineation and classification [78, 79]. There are, however, significant challenges of the MRSI approach owing to sub-optimal shim solutions, lipid contamination, poor water suppression and long acquisition times.

2.7.2 Hadamard Encoding

Instead of encoding spatial information using the phase of the MR signal, Hadamard approaches selectively align the magnetisation, $\vec{M}$, in each volume, along opposite ends of one axis across multiple acquisitions. This is done longitudinally along $\pm M_z$ or transversally across $\pm M_y$ [36]. If the manipulation of magnetisation in each volume is described by a Hadamard matrix, then it becomes straightforward to decode the individual signals arising from each volume.

For the spatial encoding of four volumes $V^T = (V_1, V_2, V_3, V_4)$, the fourth order symmetric Hadamard matrix H_4 , appears as

$$H_4 V = \begin{pmatrix} +1 & +1 & +1 & +1 \\ +1 & +1 & -1 & -1 \\ +1 & -1 & +1 & -1 \\ +1 & -1 & -1 & +1 \end{pmatrix} \begin{pmatrix} V_1 \\ V_2 \\ V_3 \\ V_4 \end{pmatrix} = \begin{pmatrix} V_1 + V_2 + V_3 + V_4 \\ V_1 + V_2 - V_3 - V_4 \\ V_1 - V_2 + V_3 - V_4 \\ V_1 - V_2 - V_3 + V_4 \end{pmatrix}, \quad (2.34)$$

where each row of the encoding matrix corresponds to a subsequent acquisition. For transverse encoding, +1 in H_4 corresponds to encoding along $+M_y$ and equivalently -1 corresponds to $-M_y$. Therefore, the first acquisition in the experiment would place the magnetisation of all four volumes along $+M_y$, a subsequent acquisition then generates oppositely phased magnetisation in the last two volumes (i.e. along $-M_y$), and so on. After applying with the encoding H_4 , each acquired signal contains information from

each of the volumes. These signal combinations can be readily decoded by applying the inverse Hadamard encoding matrix, H_4^{-1} , to the acquired data

$$H_4^{-1}(H_4 V) = H_4^2 V = 4 \begin{pmatrix} V_1 \\ V_2 \\ V_3 \\ V_4 \end{pmatrix}, \quad (2.35)$$

since Hadamard matrices have the property $H_n H_n^T = nI_n$ and can be designed symmetrically such that $H_n^T = H_n$. Thus, four independent signals (one from each volume) are recovered.

The Hadamard scheme can be extended to different numbers of encodings n , using Hadamard matrices H_n , where $n = 2^k$ for all positive integers $k = 1, 2, 3, \dots$. Upon decoding the data from an n^{th} order Hadamard scheme, the signal from each volume is obtained, weighted by factor n . Thus, signal averaging of each individual location is an inherent property of Hadamard encoding.

For the case of $n = 2$ the encoding simplifies considerably. The combined acquired signals, using a H_2 matrix scheme, look as

$$H_2 V = \begin{pmatrix} +1 & +1 \\ +1 & -1 \end{pmatrix} \begin{pmatrix} V_1 \\ V_2 \end{pmatrix} = \begin{pmatrix} V_1 + V_2 \\ V_1 - V_2 \end{pmatrix}. \quad (2.36)$$

To decode this, applying the inverse Hadamard matrix H_2^{-1} is equivalent to firstly adding together both acquisitions and secondly subtracting them, revealing independent signals of V_1 and V_2 , respectively. Fig. 2.8 shows the modulation of magnetisation required for one-dimensional Hadamard encoding and subsequent decoding of signals from either volume.

Practical Implementation

Hadamard encoding was proposed as an acquisition strategy to improve upon the signal leakage observed between successive slices when using 3-D Fourier transform imaging [80]. The technique had previously been discussed by Maudsley as a method to simultaneously acquire information from multiple ‘lines’ or planes within an object [81]. Hadamard encoding has since been proposed in the context of multi-voxel MRS localisation using both longitudinal [82, 83] and transverse encoding [84]. Longitudinal

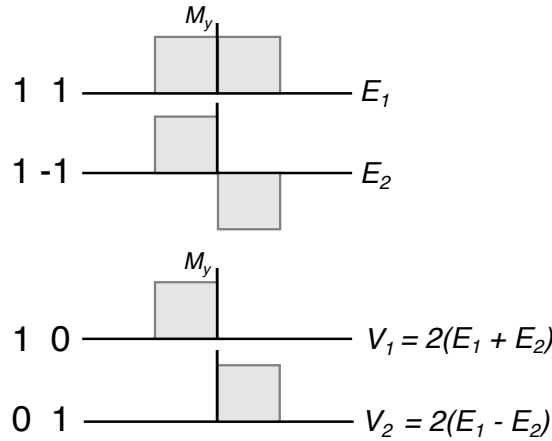


Figure 2.8: Modulation of the magnetisation required to perform one-dimensional Hadamard encoding in the transverse plane. Two experiments are run (E_1 and E_2) which excite two frequency separate frequency bands. E_2 introduces a π -phase shift over the frequency band corresponding to V_2 . Both volumes (V_1 and V_2) are separately recovered after addition and subtraction of the preceding signal from either experiment.

Hadamard encoding suffers in practice due to the fact that a single uninverted volume contains signal from regions outside of the volumes of interest that experience no inversion, thus reducing the number of acquirable volumes to $(n - 1)$ [36, 85]. Transverse Hadamard encoding, on the other hand, allows the acquisition of n volume elements from n acquisitions.

Gonen *et al.* combined transverse Hadamard encoding with 2-D chemical shift imaging to create a hybrid 3-D spectroscopic imaging approach [86]. In that work, they demonstrated that four Hadamard encoded 2-D CSI grids could be acquired with identical spectral quality and maximally efficient signal-to-noise ratio. De Graaf *et al.* have shown that n -fold transverse Hadamard encoding is possible in a double spin-echo experiment using two groups of n refocussing pulses. By modifying the phase of the refocussing pulses by 90° across subsequent acquisitions, the magnetisation profile M_y could be fully inverted [87]. An alternative to this approach to transverse encoding is the modification of the excitation pulse [80, 84]. Dreher *et al.* have used a multi-frequency excitation pulse, which is a superposition of four single-frequency sinc pulses, to perform transverse Hadamard encoding [88].

Multi-band RF-based localisation

One fundamental limitation of the Hadamard-based approach is the upper limit to the number of volumes practically encodable. Localisation requires the use of several frequency selective RF pulses or more commonly a single RF pulse capable of spatially selective localisation over multiple frequency bands [89]. Since the amplitude of an RF pulse is proportional to the number of bands it excites (or inverts), the RF peak power requirement for an n -band pulse goes as n^2 . This typically limits the technique to $n \leq 8$ [36].

Multi-band pulses can be readily generated from an existing single band pulse. Using the shift theorem of the Fourier transform $\tilde{h}(\omega)$ of $h(t)$

$$\begin{aligned} \text{if } h(t) &= e^{-i2\pi\Delta\omega t} g(t), \\ \text{then } \tilde{h}(\omega) &= \tilde{g}(\omega + \Delta\omega). \end{aligned} \quad (2.37)$$

Thus, a frequency selective pulse $g(t)$, centred on ω , can be shifted by an off-resonance frequency amount $\Delta\omega$, through multiplication with a complex exponential. In practice, this results in a linear phase increase through the duration of the RF pulse. A multi-band pulse, P_n , with bands centred on ω_k where $k = 1, 2, \dots, n$, can then be generated by superposition of shifted versions of the original pulse P_0 , such that

$$P_n(t) = \sum_{k=1}^n P_0(t) e^{-i2\pi\omega_k t} \quad (2.38)$$

Each excitation (or inversion) band may behave identically to that of the single pulse, provided that there is sufficient separation between the bands. Steffen *et al.* have described the small Bloch-Siegert style shift that occurs when irradiating at a nearby off-resonant frequency, when band separation is too close, leading to a degradation of the ability to perform full inversion [90]. The interaction between inversion bands has been shown to be a particular problem for adiabatic pulses because of the generation of a new effective field, although the adiabatic condition can be modified for multiple inversion in Hadamard encoded acquisition schemes [91–93]. For applications where a large number of bands are required, techniques exist to reduce the amount of RF power required, such as temporally shifting (or cascading) the individual single band

pulses, i.e. $P_0 \rightarrow P_0(t + \tau_k)$, before generating the multi-banded pulse [94]. This has the advantage of ensuring peak power of P_n is approximately equal to P_0 [95].

Voxel-bleed, chemical shift and ultra-high field

The large intrinsic point spread function (PSF) blurs signal across the whole FOV in Fourier-based chemical shift imaging [96]. Signal leakage or 'voxel-bleed' in Hadamard encoding is small and can be attributed to overlapping magnetisation profiles between slices [84]. Voxel-bleed is, thus, dependent on limitations of RF- and gradient strength. Goelman *et al.* describe that the percentage of signal contribution arising from outside a particular slice can reach 17% (for a 1kHz bandwidth, 5 ms pulse at 3 T) [97]. These authors showed that by adding a small offset to the VOI of each slice, to account for the imperfect magnetisation profile of each pulse, voxel-bleed artefacts arising from addition and subtraction of contiguous slices can be reduced. Voxel-bleed can be particularly problematic for MR spectroscopy where subtle differences in chemical composition between adjacent voxels are expected.

A particular problem faced when applying Hadamard encoding to multi-voxel spectroscopy is that of chemical shift displacement (CSD) artefact. Since encoding is based on RF localisation, large gradient strengths (and hence low peak B_1 power), are required to reduce the displacement of metabolite signals across the slice direction. The problem is further exacerbated in Hadamard encoding with the use of high power multi-band inversion or excitation pulses, such that gradient strengths need to be made weaker in order to allow for reduced pulse bandwidths, which subsequently leads to larger CSDs. Cohen *et al.* have demonstrated a 3-D combined longitudinal and transverse Hadamard approach using cascaded multi-band pulses at 3 T [98]. Cascading reduces the overall B_1 requirement for each slice and, thus, allows for an increase in the bandwidth available per slice. They report a CSD of 0.3 mm/ppm at 3 T (or around 35-fold less than an equivalent PRESS sequence) as well as 8-16% less signal bleed than a similar CSI acquisition. Pulse cascading has also been demonstrated by Tal *et al.* at 3 T in a fully 3-D longitudinal Hadamard encoded implementation [95]. This resulted in a bandwidth of 1.8 kHz per voxel and CSD of 0.9 mm/ppm [95]. At

7 T the RF power required to achieve the same flip angle is around twice that at 4 T [57], thus gradient strengths must be made proportionally smaller than at lower field strengths. Goelman *et al.* have proposed a combination of two techniques to combat the large CSD at 7 T for Hadamard encoding [99]. Their proposed technique amounts to a reduction in peak B_1 requirement through pulse cascading and multiplexing the acquisition of n of slices by dividing them into m 'sub-slices' acquired within a single TR [100]. The localisation of these sub-slices requires reduced B_1 power and, thus, CSD of 0.5 mm/ppm can be achieved at 7 T [99]. It is noted, however, that cascaded pulse approaches will introduce differential T_2 -weighting based on the order the slice profiles are excited and this effect can accumulate across dimensions. In addition, cascading relies on the fact that truncation of individual shifted pulses before addition will not have a significant effect on the individual slice profiles.

3

Brain Metabolism: Biological Context

In this chapter, the literature surrounding the questions of brain metabolism in this thesis, are reviewed. In particular, the first half of this chapter (Section 3.1) will deal with the haemodynamic, neuronal and neurochemical metabolic changes in the brain upon functional activation. This ultimately is a review of fMRI-BOLD signals and their link to excitatory synaptic activity. Mechanisms of coupling between neurons and the vasculature are also reviewed. This section also deals with the observation of negative BOLD responses and the hypotheses explaining their mechanism. The second half of this chapter (Section 3.3) is concerned with the the IDH-mutation in human glioma. This includes the production of 2-hydroxyglutarate, its implications for the cancer cell and the current research on metabolic reprogramming in IDH-mutated tumours.

3.1 What generates BOLD responses?

Functional activity, energy metabolism and blood flow are linked in the brain. It is this fundamental idea that underpins functional MRI (fMRI); leading to >40,000 fMRI-related journal articles to date (according to a recent estimate [101]). A century before fMRI began, studies already pointed to an association between elevated blood responses and functional activity in the brain [102–104]. Yet even today, the mechanism of this interaction, and in particular the nature of the link between neural activity and haemodynamic control in the brain, is still an active area of research. In this section, the current, and often contentious, understanding of this area is reviewed.

Ogawa *et al.* first demonstrated the MR contrast mechanism that forms the basis for the majority of functional imaging studies today [105–107]. In their seminal work on rodent brains, they observed that an increased concentration of deoxyhaemoglobin (dHb) in the blood generated signal dropout using a gradient-echo sequence [105]. This blood oxygenation level-dependent (BOLD) reduction in signal occurred around

vessels due to an increased T_2^* -dephasing from dHb [105, 107] and also changed during an increase in metabolic demand [106]. BOLD contrast was also observed in cat cortex under anoxia [108]. The large paramagnetic susceptibility of dHb [109], thus acts as an endogenous MR contrast agent in the brain. BOLD contrast was then demonstrated in human volunteers presented with a visual stimulus [110, 111] or during a finger-tapping task [112], marking the birth of fMRI.

3.1.1 Increased metabolic demand

In fMRI-BOLD studies, the MR signal amplitude *increases* in active brain regions relative to a baseline level, reflecting a reduction in T_2^* -decay. Counterintuitively, this is generated by an increase in the relative blood oxygenation in active regions. Fox and Raichle had previously shown, using positron emission tomography (PET), that a small increase in metabolic rate of oxygen (CMRO₂) (~ 5%) is observed in active brain regions accompanied by a much larger increase in local cerebral blood flow (CBF) (~ 50%). This generates the surplus of oxyhaemoglobin in these regions and the BOLD signal increase relative to baseline. fMRI-BOLD is therefore a measure of the discrepancy between changes in Δ CMRO₂ and Δ CBF within a brain region. Changes in cerebral blood volume (Δ CBV)¹ may also generate BOLD signal changes [114, 115]. The large discrepancy between CBF and CMRO₂ led researchers to question the metabolic need for this functional hyperaemic response during neural activity.

Initial studies provided evidence for an increased anaerobic metabolism during functional activity. Almost four decades ago, Sokoloff *et al.* proposed a link between increased glucose utilisation and neural activity in the brain [116] and found that activation induced blood flow responses [117]. Although oxygen utilisation and its delivery appeared uncoupled during activation [118], Fox *et al.* observed an increase in the metabolic rate of glucose (CMRglu), which roughly matched the increased supply through the CBF [119]. In addition, many studies found increases in extracellular concentrations of lactate during periods of stimulation [120–122]. Together, these findings suggested that increases in oxidative phosphorylation during functional

¹Changes in cerebral blood volume (CBV) are often equated to CBF changes in the literature via a power law relationship first demonstrated by Grubb *et al.* [113].

activity are small compared to those of anaerobic glycolysis, since the latter ultimately produces lactate. Thus the hypothesis emerged, that anaerobic glycolysis may support functional activity in the brain and that CBF increases are driven by this altered metabolic demand.

Many studies have since called this hypothesis into question and are unable to repeat the finding of such a low rate of oxidative metabolism. Using a mathematical model of flow responses and blood oxygenation, Mintun *et al.* suggested that during functional activity, enough oxygen is present in the blood supply to support oxidative pathways without a need for a CBF increase [123]. In fact, CMRO₂ has been shown to increase much more than originally reported [124]. A meta-analysis of 20 publications found that the average Δ CMRO₂ increase during activation was as high as $20 \pm 13\%$ [125]. Differences in Δ CMRO₂ in the BOLD literature may have arisen from incorrect assumptions in the relationship linking changes in CBF to CBV [126, 127]. It is also clear that oxidative phosphorylation is a more efficient energy source, producing 30 adenosine triphosphate (ATP) molecules per glucose compared to anaerobic glycolysis which only produces 2 ATP. As a result, even small increases in CMRO₂ (~5%) were estimated to result in oxidative pathways generating 60% of the ATP derived from glucose [128].

The energy required to sustain functional activity may, therefore, still largely arise through oxidative metabolism. Further support for this claim is that the observed increases in lactate were shown to only partly account for glucose utilisation through increased glycolysis [129]. Mangia *et al.* suggest that lactate increases must equilibrate with pyruvate levels in the steady state, and as such, flux through oxidative pathways must necessarily increase [130]. Nevertheless, it is apparent that CMR_{glu} is enhanced during functional activation. The metabolic consequences of this, and its link to neuronal activity, must be addressed at the molecular and cellular level.

The astrocyte-neuron lactate shuttle hypothesis (ANLS)

One hypothesis in the literature, which may account for enhanced glycolytic activity observed during activation, has been proposed. This hypothesis suggests a tight coupling exists between glycolytic metabolism in astrocytes and oxidative metabolism

in neurons, which is mediated via lactate [131]. A pivotal study by Sibson *et al.*, using infused ^{13}C -labelled NMR of glucose in rat brain, found a 1:1 stoichiometric relationship between the rate of glutamine-glutamate cycling in astrocytes and the oxidative metabolism of glucose through the TCA cycle [132]. This stoichiometry suggests that for each molecule of glutamate cycled between neuron and the astrocyte, the complete oxidation of one molecule of glucose is required, thereby linking excitatory neuronal activity via glutamate release to metabolic demand [133]. Previous to this finding, Pellerin and Magistretti discovered that the presence of extracellular glutamate stimulates anaerobic glycolysis in glial cells to power the uptake of glutamate and cotransport of Na^{2+} as well as the glutamine synthase reaction, consequently producing lactate [7]. This observation was combined with the ^{13}C observations to form the astrocyte-neuron lactate shuttle (ANLS) model.

The ANLS model states that glucose is taken up preferentially by astrocytes where it is anaerobically metabolised into $2 \times \text{ATP}$ and $2 \times \text{lactate}$ [125], providing the exact amount of ATP for the glutamate uptake in the astrocyte (cotransport of Na^{2+}) and glutamine synthesis. The produced lactate is then shuttled to the neuron where it provides an energy substrate for oxidative metabolism, generating $34 \times \text{ATP}$ [134]. The large amount of energy from oxidation is used to power neuronal processes, which support neurotransmission and neuronal firing.

Lactate is, therefore, both a product of anaerobic glycolysis and also a substrate for further aerobic metabolism. The use of lactate as an energy substrate is achieved via conversion of lactate to pyruvate (via lactate dehydrogenase (LDH)) and subsequent entry into the TCA cycle, which may occur predominately in neurons. Thus, lactate has been suggested to play a key role in intracellular metabolism in the brain through shuttling of lactate from neuron to astrocyte, where it is then oxidised - the lactate shuttle hypothesis [135].

Support for the ANLS hypothesis of neurometabolic coupling has been demonstrated in many studies [136] (some of the findings are reviewed by Mangia *et al.* [128]). However, aspects of ANLS are controversial and Pellerin *et al.* published a

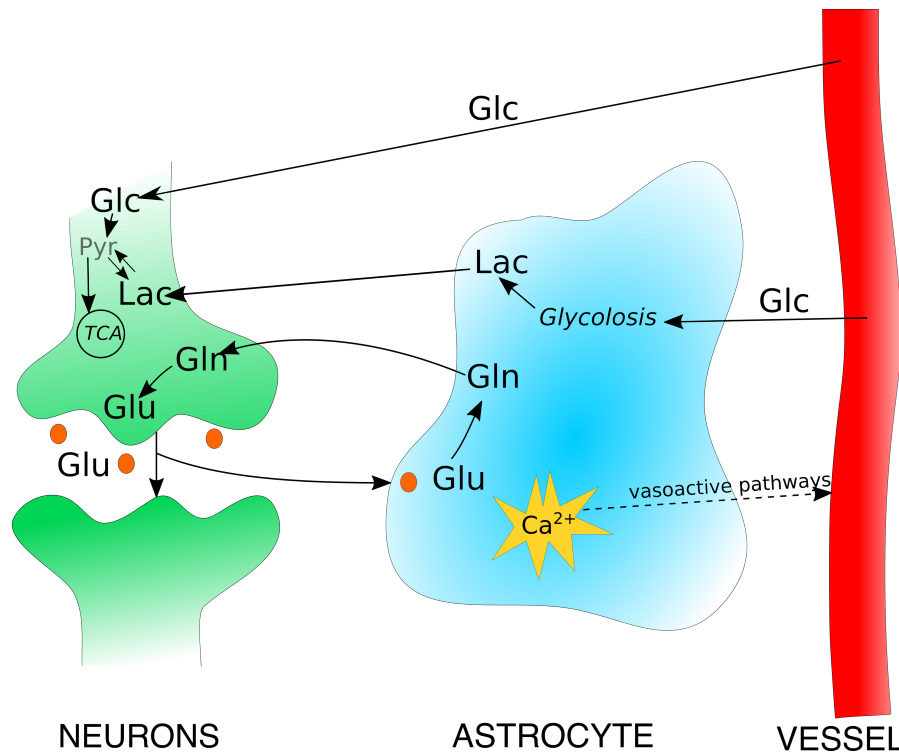


Figure 3.1: A potential mechanism of interaction between neurons and astrocytes which may sustain excitatory activity according to the ANLS hypothesis. Glutamatergic (Glu) activity at synapses is taken up by astrocytes where it may potentially trigger release of vasodilatory molecules through the calcium cascade $[Ca^{2+}]_i$. In this model, neurotransmitter cycling leads to uptake of glucose to support functional activity which may be fully metabolised in the TCA cycle of neurons, thus requiring shuttling of lactate (Lac) from astrocytes.

comprehensive review examining such issues [137]. In particular, it has been questioned whether lactate can act as a neuronal energy substrate during activation [138, 139]. There are also implications of the ANLS hypothesis on energy use in the brain. Firstly, given the level of oxidative metabolism *in vivo* and the ANLS model of glucose metabolism, it was calculated that ~85% of total glucose oxidation at rest is coupled to glutamate-cycling [140]. Attwell and Iadecola point out, however, that such a model fails to account for other forms of ATP production such as in mitochondria and that glial cells use significant energy to maintain resting potentials [141]. The authors suggest that the estimation of > 80% of glucose oxidation used to support glutamate cycling, is a large overestimation. The ANLS model may explain the imbalance of glycolytic metabolism during functional activity, through the rapidity of glycolytic pathway in astrocytes to initially support glutamate uptake [142]. However, Öz *et al.*

have measured a substantial *oxidative* component of metabolism in glial cells (30%) [143]. Thus, in order to bring the ANLS model in line with this finding, a component of anaerobic glycolysis must also be accounted for in neurons, yet still retain a 1:1 stoichiometry with glutamine-glutamate cycling [144]. Notably, the ANLS theory has also been modified to include contributions from neurotransmitter cycling of GABA (a derivative of glutamate) [144].

In any scenario, there exists a strong relationship between glucose oxidation in neurons and astrocytes and neuronal activity following glutamatergic release. During periods of functional activation, Schulman *et al.* have proposed that a rapid reliance of glycolysis for glutamate clearance in glial cells may generate a surplus of lactate that is not fully oxidised, thus, leading to an efflux into the blood [125]. Lactate increases have also, interestingly, been shown to generate functional hyperaemic responses via an increase in the NADH/NAD⁺ ratio, activating vasodilatory signalling pathways [145, 146]. The build-up of lactate during functional activity may simply act to promote the effect of vasodilatory molecules already present extracellularly [147]. Enhanced glycolysis in astrocytes may however result in an elevated CBF similar to that observed during BOLD responses. For a thorough understanding of the generation of the BOLD response, it is necessary to understand how neural activity, specifically excitatory synaptic activity, is coupled to blood flow in the brain.

3.1.2 BOLD contrast reflects excitatory synaptic activity

Evidence for a link between the BOLD response and increased synaptic activity can be gleaned from a consideration of cortical organisation. Measurements of vascular density across cortical layers have revealed increased vascularisation in regions of higher synaptic (rather than neuronal) density (corresponding to cortical layer IV) [148, 149].

In compelling work carrying out electrophysiological recordings during BOLD responses, Logothetis *et al.* have confirmed that BOLD responses do indeed reflect underlying neural activity [150]. More precisely, using recording techniques, the *synaptic* nature of BOLD responses has been exquisitely revealed. Extracellular field potentials (EFPs), which contain weighted averages of the voltages across many neural

cells, can be measured using implanted electrodes (reviewed by Logothetis *et al.* [149]). Under correct experimental conditions, EFPs comprise of contributions from the action potentials of several firing neurons and also activity from synaptic events. These signals are separated by band-passing the EFP with the appropriate frequencies revealing the multiple-unit spiking activity (MUA) and the local field potential (LFP), which reflects the synaptic activity amongst other slower responses [149]. LFPs have, therefore, been used as an indicator of local sub-threshold processing or input signals. Whereas MUAs reflect to a larger degree the neuronal spiking or action potentials.

Logothetis *et al.* found that BOLD responses in the anaesthetised monkey brain reflected an increase in neural activity through an enhanced EFP signal [150]. Similarly, other groups also reported increases in spiking frequency during BOLD responses [151, 152]. By using the measured LFP and MUA, Logothetis *et al.* were able to show that both signals were good predictors of BOLD responses, though the LFP was significantly better [150]. BOLD signals thus reflect more local synaptic processing than neuronal spiking as originally presumed. Mathiesen *et al.* have shown, in a system in which synaptic activity is present but spiking inhibited, that CBF and LFPs are both increased [153]. Therefore the literature provides clear evidence that blood flow, and hence the BOLD signal, is correlated with increased synaptic activity.

3.1.3 Mechanisms of neurovascular coupling

Although the previous studies confirm a close relationship between the LFP (or local synaptic processes) and CBF, precisely how this neurovascular coupling is apparent during functional activity is still unclear. Increasing evidence points to the role neurotransmitters may play in the control of CBF [147]. Several discoveries have highlighted the importance of *astrocytes* once more, not only energetically (see ANLS hypothesis above), but also for the control of CBF through release of vasoactive molecules. This field of research is, however, contentious; conflicting reports are present in the literature and as such the hypotheses discussed are still under active scrutiny.

Interestingly, the role of astrocytes in mediating neuronal activity and blood flow was recognised in 1895 by Ramón y Cajal [154] based on observation of their proximity

to neuronal synapses and their characteristic ‘end-feet’, which are located close to blood vessels. They are, therefore, uniquely positioned to mediate neurovascular control. The link between synaptic activity, astrocytes and CBF hinges on the finding that glutamatergic activity can generate intracellular calcium rises ($[Ca^{2+}]_i$) in astrocytes [155] (for review see: Bazargani & Attwell [156]). For example, one study noted that inhibition of glutamate receptors (mGluRs) on astrocytes reduced $[Ca^{2+}]_i$ and also vessel dilation in rats [157]. The biochemical result of astrocytic $[Ca^{2+}]_i$ is the production of various substances derived from arachidonic acid (AA), which have vasoactive effects on the smooth muscle surrounding arterioles; thereby generating a dilatory (PGE₂ [158], EET [159]) or constrictory (20-HETE, [159, 160]) response on nearby vasculature.

Vascular responses to astrocytic $[Ca^{2+}]_i$ may, therefore, be bidirectional. Attwell *et al.* suggest the ultimate direction of response could be mediated by nitric oxide (NO) [147], an important vasodilatory molecule, which has been shown to mediate, but not fully control, the local blood response [161]. For instance, release of NO has been shown to inhibit the production of AA derivatives of vasoconstriction (20-HETE) [162]. In the retina, Metea and Newman showed that NO has a vasoconstrictive effect [159]. The local availability of oxygen is claimed to predominantly impair the synthesis of both NO and 20-HETE, leading to a largely vasodilatory response [147, 163]. This has implications for the generation of BOLD responses; during which local oxygen concentration dips during the onset of activation, therefore potentially mediating a dilation in the local microvasculature and increase in CBF.

The evidence for astrocytic involvement in neurovascular coupling is convincing (see review: [164]), however a temporal delay between blood response (~ 1 s) and $[Ca^{2+}]_i$ dynamics (1s – 6s) has been noted (although newly discovered rapid mechanisms may explain this discrepancy [156]). Aside from astrocytic control of blood flow, recent work has pointed at the role of pericytes (small contractile cells positioned on capillaries [164]) for the control of blood flow, though further evidence may be needed to establish their connection with functional imaging [165]. It must also be noted that current evidence for astrocytic release of neurotransmitters is controversial since

data have largely come from artificially controlled *in vitro* experiments, whereby several cellular mechanisms may be impaired [166]. Additionally the role of direct neuronal control on vascular tone cannot be ruled out [167]. There is a body of evidence that supports a significant role for GABAergic interneurons as mediators of vascular control [168] and this will be discussed in the section on negative BOLD signals (Section 3.2).

3.1.4 Insight from *in vivo* MRS

Since the inception of fMRI, a handful of studies have used *in vivo* ^1H -MRS to investigate BOLD responses in humans. Early studies noted rises in lactate concentration after minute-long stimulation, often with a slow return to baseline [169–171]. Over shorter timescales (< 12 s), Mangia *et al.* observed initial decreases in lactate using a long-TE PRESS sequence [172]. These decreases were attributed to an initial period of hypoglycaemia whereby lactate was used up as an energy substrate before glucose is restored by the blood response.

The sensitivity of MRS for measuring small metabolite changes is dependent on the field strength, as discussed in Ch. 2. For instance, Boucard *et al.* reported no functional metabolite changes at 1.5 T after visual stimulation and had issues with spectral contamination [173]. At higher field strengths, metabolite detectability increases. For instance, Mullins *et al.* were the first to report glutamate increases in regions responsive to a pain stimulus [174] at 4 T. Glutamate increases were suggested to be evidence for increased neurotransmission and Glu-Gln cycling upon stimulation. At 7 T, Mangia *et al.* have shown the considerable advantages for functional spectroscopy at high field strength, resulting in a sensitivity for changes of $\pm 0.2 \mu\text{g/mol}$ [175]. Functional MRS studies must, however, account for the line-narrowing of spectra, which occurs as a result of the increased T_2^* and can artificially change metabolite estimation [176]. Schaller *et al.* reproduced findings of increased glutamate ($\sim 4\%$) and lactate ($\sim 20\%$) at 7 T during visual stimulation and were able to detect a total of 16 metabolites using a short-TE SPECIAL sequence [177]. Mangia *et al.* similarly reported increases in lactate ($\sim 23\%$) and glutamate ($\sim 3\%$) in addition to aspartate decreases ($\sim 15\%$) at 7 T [130]. Lactate increases were interpreted as increases in oxidative metabolism, partly

through increased pyruvate levels (see above section). Interestingly, glutamate and aspartate are linked via the malate-aspartate shuttle (MAS), a pathway necessary to restore NADH/NAD⁺ for oxidative metabolism [178]. Therefore, glutamate changes measured by MRS may not represent neurotransmission directly, but rather the effect of increased oxidative metabolism. The authors have suggested that opposite changes in glutamate and aspartate reflected increased oxidative metabolism. A comprehensive review of the mechanism underlying observed glutamate changes during functional activity can be found in Ref. [179].

Essentially, an increase in pyruvate metabolism would in turn lead to increased acetyl-CoA in mitochondria. Two enzymes compete for acetyl-CoA, catalysing aspartate or citrate production (via mAAT and CS, respectively). Under increased oxidative metabolism, citrate production is preferred thus leading to a reduced availability of aspartate. Through MAS, the uptake and eventual breakdown of glutamate in the mitochondria is then reduced [179]. Similar conclusions were recently drawn by Bednařík *et al.*, who detected functional increases in glutamate (~30%) and lactate (~3%), with significant decreases in aspartate (~5%) and glucose (~16%) during visual stimulation at 7 T [180]. *In vivo* MRS at 7T is thus uniquely positioned to measure functional changes in neurotransmitters levels, in particular glutamate, although a cautious interpretation of the results must be applied.

It appears that functional changes in lactate and glutamate, with reductions in aspartate, may reflect increases in the oxidative metabolism of glucose rather than increased neurotransmitter cycling.

3.2 Negative BOLD Responses

Conventional BOLD signals, as discussed above, reflect functional hyperaemia following excitatory synaptic activity. Over the years of fMRI-BOLD, however, many studies have pointed to observable *reductions* in the BOLD imaging signal in various brain regions during tasks. Yet, the mechanism driving these signal reductions remains elusive. Reductions in the BOLD response were observed in unstimulated regions of cortex during visual stimulation [181–184], visual attention tasks [185–188], somatosensory

cortex [189, 190], during motor stimulation [191–195], across multiple brain regions [196] and even the default mode network (DMN) [197]. The unique aspects of these observed negative BOLD responses (NBRs) are that they are robust and sustained over long stimulus durations. As a result, these responses are functionally different from the ‘initial dip’ observed commonly preceding the conventional (or positive) BOLD response [198] (although the initial dip is not observed in all cases and its physiological mechanism is contentious [199, 200]). Relatively little research has been conducted into understanding the nature of NBRs compared to conventional positive BOLD responses (PBRs). Recent evidence suggests that regions of NBR may reflect neuronal suppression, therefore fMRI-BOLD may capture a more subtle and detailed picture of the brain’s response to task. Investigation of the link between blood flow, metabolism, and neuronal activity in NBRs is, therefore, a crucial avenue of research. This section begins with a review of the possible mechanisms of NBRs.

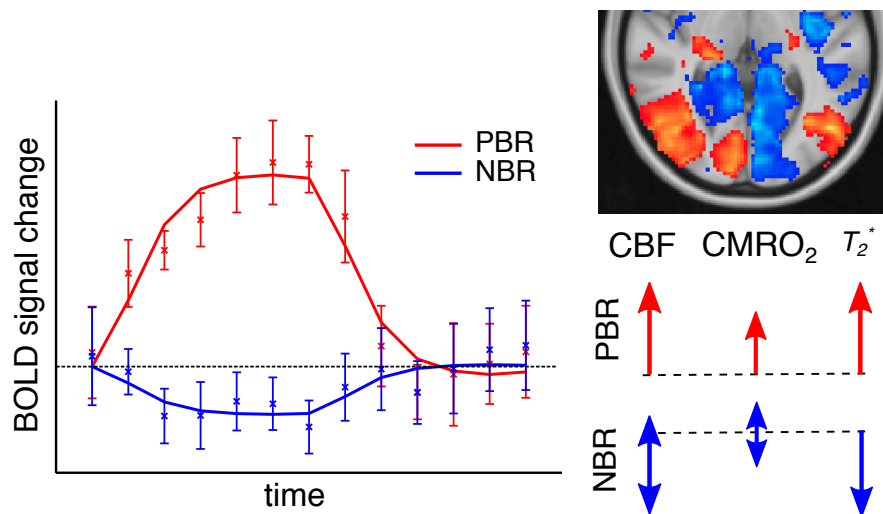


Figure 3.2: Positive and negative BOLD responses generated using the visual stimulus in Chapter 5. **Left:** Sustained reductions in BOLD signal amplitude occur in the negative BOLD response and are smaller in magnitude than the positive response. **Right:** Possible alterations of CBF and metabolic rate of oxygen (CMRO₂), which lead to responses of either type. For example, negative BOLD may be generated by a CBF decrease of larger relative magnitude than the change in CMRO₂.

3.2.1 Neuronal suppression rather than blood-steal

From a signal perspective, a reduction in BOLD reflects a reduction in the transverse magnetisation decay rate T_2^* , arising from a local deoxyhaemoglobin content increase. Theoretically, this scenario may arise from (1) an increase in metabolic demand (CMRO_2) greater than any change in cerebral blood flow (CBF) or (2) a CBF decrease greater than any change in CMRO_2 . It is well understood that the PBR is a result of an overcompensatory increase in CBF in active regions characterised by increases in CMRO_2 . Following this explanation, NBRs may also be generated by a CBF *decrease* which overcompensates for any reduction in CMRO_2 . However, an alternative coupling between CBF and CMRO_2 may serve to explain the generation of NBRs (see Fig. 3.2). For example, there may be zero net CBF change in NBR regions while simultaneously CMRO_2 may increase. Alternatively, CBF in such regions may *undercompensate* any increase in CMRO_2 .

A handful of hypotheses have been proposed that serve to explain the sustained negative BOLD response. One of these, the blood-steal hypothesis, was put forward by Harel *et al.* after observing NBRs during visual stimulation of cat brain in regions characterised by a delayed decrease in CBV (and hence CBF) compared to PBR [182, 201]. Under the assumption that total blood flow in the brain remains constant, Harel *et al.* proposed that a diversion of CBF to regions of higher metabolic demand may occur, leaving adjacent or surrounding areas with a net CBF reduction, resulting in an NBR. Mathematical modelling of localised flow increases through arterioles also predicted a reduction in blood flow and decrease in oxyhaemoglobin in surrounding regions, which could partially account for previously observed NBRs [202]. The diversion of blood is an appealing explanation, although the majority of recent studies have failed to support this hypothesis.

Some of the most convincing evidence against blood-steal is from studies where NBR is evoked contralaterally to PBR. Smith *et al.* noted that the two cerebral hemispheres have largely independent blood supplies, via the carotid arteries, thus the observation of contralateral NBR to PBR in primary visual cortex (V1) suggested negative BOLD signals could not be explained purely haemodynamically [188]. The

same conclusion is also strongly supported by observations in motor regions where contralateral NBRs occur at a greater distance from areas of activation [191, 193]. An alternative hypothesis was proposed: ‘blood-sharing’, whereby local activation in one region could control blood flow in other regions [188]. Yet, evidence for both the mechanism and requirement for such long range neuronal control of vasculature is lacking. Furthermore, although the timing of the CBV response lagged the NBR in the study by Harel *et al.* [182], Liu *et al.* found the temporal dynamics of NBR could not arise from CBF change that lagged the onset of PBR in the active region [195]. Shmuel *et al.* also found very tight coupling between the temporal dynamics of NBR and PBR in humans [183], suggesting similar underlying mechanisms coupling CBF to CMRO₂ in PBR and NBR, although a haemodynamic component common to both signals may still exist and explain some of this similarity.

A second hypothesis for observed NBR is that there might be an uncoupling between blood responses and metabolic activity, resulting in increased metabolism (CMRO₂) not matched by any increase in CBF. Several studies have provided evidence contrary to this proposal, primarily through the observation that CBF in fact decreases in NBR regions. In the study by Shmuel *et al.*, CBF was observed to decrease in negative BOLD regions (~10%) [183]. Using the Davis model [126], they found CMRO₂ was also significantly decreased in NBR compared to baseline, albeit in a relatively smaller fraction (~5%) than the reduction in CBF. The lower metabolic rate of oxygen consumption was interpreted as reduced activity (below baseline) which in turn generated a reduction in CBF. In novel work by Pasley *et al.*, a larger relative increase in CMRO₂ and CBF was required in PBR regions generated on negative BOLD baselines compared to those evoked under resting conditions [203]. This larger metabolic difference in CMRO₂, to match the change in CBF, was interpreted to suggest that the underlying NBR was not purely haemodynamic in origin, since the coupling between CMRO₂ and CBF is maintained between NBR and PBR. Stefanovic *et al.* also performed CBF and CMRO₂ measurements, in ipsilateral regions of induced inhibition during a hand-grip task, and noted a decrease in metabolic activity (CMRO₂) in these regions

combined with a much larger decrease in CBF [193]. It is, therefore, apparent that metabolic activity decreases during NBR, but this is met by a greater decrease in CBF.

While it is possible that a fraction of the observed NBR may occur due to translation of blood between regions [204], or vascular dynamics of local blood flow [202], the majority of evidence points to suppression or reduction in neuronal activity. In seminal work on NBR in monkey brain, Shmuel *et al.* used electrophysiological measurements of local neuronal activity (in a similar vein to Logothetis *et al.* in the PBR [150]) and found a *reduction* in neuronal activity as measured by reduced amplitudes of LFP, MUA and neuronal spiking in NBR regions [205]. The decrease in neuronal activity was a better predictor of NBR timecourses than the adjacent PBR and occurred immediately after stimulus onset. Devor *et al.* also showed in rat brain that deactivation of neurons (marked by LFP and MUA decreases) surrounding active regions of increased blood oxygenation was accompanied by vasoconstriction and deoxygenation [206]. This lends further convincing evidence that the NBR could be a marker of neuronal deactivation and could be mediated by inhibition.

In a recent combined study, using fMRI-BOLD in combination with EEG and CBF measurements, Mullinger *et al.* [207] reported a difference in the coupling between CMRO₂ and CBF changes, compared to previous studies using updated coefficients in the Davis model [207]. This coupling was different across NBR and PBR regions, suggesting that different underlying neuronal processes link CMRO₂ to CBF in deactivated regions. In addition, they found that an increase in the power of mu-oscillations (measured using EEG) coincided with increased amplitude of NBR. This is interesting, since mu-oscillations have previously been linked to inhibitory control [208].

Further evidence for the neuronal suppression hypothesis comes from the neuroscience literature. Kastrup *et al.* showed that sensory stimulation produced ipsilateral negative BOLD in primary somatosensory cortex (S1) which corresponded to measures of increased threshold of perception, suggesting that NBR serves to shape perception [190]. Heinemann *et al.* have shown that peripheral generation of NBR in the visual system is increased when distractor stimuli were presented with no effect on the foveal representation in the PBR [209]. Suppression of unattended locations has been shown

in numerous further studies, e.g. [1, 210, 211]. NBR has been also shown to carry stimulus specific information about stimulus position [212] indicating that negative BOLD suppression may finely tune the visual percept. Evidence, therefore, suggests that NBR serves to improve perceptual accuracy through a decrease of neuronal activity in unattended regions.

3.2.2 Role of synaptic excitation and inhibition

It is clear that glutamatergic (or excitatory) synaptic activity leads to increased BOLD responses. If NBRs are a reflection of increased inhibitory activity, they may potentially be associated with increased GABAergic activity within a local brain network. A disruption in the excitation-inhibition balance during functional activity, may therefore lead to BOLD responses of either polarity. To confirm these hypotheses (which is investigated in Chapter 5), information is needed on the metabolism of inhibitory processes across the brain. To date, relatively little information is available on this topic (for review see [213]). Indeed, it is not yet clear if an increase in synaptic inhibition would lead to enhanced CBF and a PBR owing to an increased metabolic demand [214]. Several key studies do, however, offer insight into inhibitory processes and their link to BOLD imaging.

Metabolic costs of inhibition

In a thought-provoking study on volunteers who underwent transcranial magnetic stimulation (TMS) in motor regions to induce local inhibition, Waldvogel *et al.* observed no difference in the positive BOLD response in such ‘inhibited’ regions [215]. The researchers claimed this was because inhibition is a more energy-efficient process, and that it, therefore, does not influence BOLD significantly. However, Attwell *et al.* point out that inhibition in this work was not a measure of increased synaptic activity, but rather reduced neuronal spiking [141]. It is clear from the previous discussion that BOLD responses reflect more local synaptic processing. Integration of the total inhibitory and excitatory processes in a network may, therefore, result in a differential spiking output. It might be expected, however, that a simple reduction in the number

of excitatory inputs in a functional network (or deactivation), without a change in inhibition, would require less energy to maintain [128].

Patel *et al.* suggested that 13-15% of oxidative metabolism in neurons and glia can be ascribed to GABA neurotransmitter cycling, suggesting that inhibitory neurotransmission is of small, but considerable energetic importance compared to glutamatergic activity [216]. Both excitatory and inhibitory activity have separately been shown to increase glucose utilisation in cat brain [217, 218]. These findings suggest there may be a price to pay for increased inhibition. In the astrocyte, however, Chatton *et al.* found that response to GABA does not trigger an increased glycolytic flux, as is observed with glutamate [219]. In interneurons the story may be different. MaCasland *et al.* have observed that GABAergic interneurons are marked by a greater glucose metabolism than glutamatergic neurons [220].

From an energetics perspective, inhibitory processes may consume much less energy than excitation. After inhibitory synaptic transmission, post-synaptic fluxes of Cl^- need to be reversed. This has been estimated to require much less energy (<1%) than the post-synaptic potential restoration of Na^{2+} gradients following glutamatergic activity and can be also be achieved without the production of ATP [221, 222]. Howarth *et al.* considered a distribution of neuronal sub-types in the cerebellum and their corresponding energy requirements and concluded that the overall energy requirement for inhibitory activity (27%) is lower than that of excitation (73%) [221]. It is, therefore, probable that inhibitory processes consume less energy on the whole compared to excitation.

Compared to baseline, or brain at rest, Mangia *et al.* hypothesise that increased inhibition should ultimately reduce energy requirements given that inhibitory synapses form only a small fraction of total number of synapses (~20%), inhibitory post-synaptic potentials require little energy and the reduction in spiking activity of excitatory neurons following inhibition may outweigh any increased energy demand from post-synaptic potentials [128]. Regions of active inhibition may, therefore, appear as negative BOLD regions, through a drop in metabolic demand (as observed through decreased CMRO_2) in fMRI experiments. Yet this does not explain why the observed CBF

decreases (as reported above) in negative BOLD regions. Lauritzen *et al.* have recently published a comprehensive review of this problem [223]. For potential mechanisms, it is necessary to once again examine the role of intracellular calcium dynamics.

3.2.3 GABA, interneurons and vascular control

Could a speculative increase in extracellular GABA concentration, through increased functional inhibition, lead to a reduction in blood flow? Interestingly, Caesar *et al.* reported a cessation in spiking and no CBF increase in cerebellar cortex of rat under conditions of tonic inhibition from a GABA agonist, although the LFP was increased [224]. Additionally the study reported a block in neuronal $[Ca^{2+}]_i$. Reduction in intracellular $[Ca^{2+}]_i$ in Purkinje neurons has also been shown following GABA-mediated inhibition [225]. These findings are important since they suggest that inhibitory synaptic activity can suppress $[Ca^{2+}]_i$ and, therefore, prevent release of vasoactive substances (e.g. NO) potentially leading to reduced CBF [226]. It is noted, however, that different neuronal types, across the brain, have vastly different reported vasoactive properties and as with all studies, therefore, findings may be region-specific [214].

Cauli *et al.* have investigated the role interneurons may play in neurovascular coupling [168]. They revealed a subset of GABAergic interneurons that are associated with vasoactive control and that innervation of a single interneuron was enough to induce dilation or constriction on nearby vessels depending on the neuronal subtype. Buzsáki *et al.* speculate that because of the role interneurons play in spiking integration, they have the potential to exert influence on vasculature in a manner dependent on the underlying activity [213]. Therefore, excitation of interneurons that release GABA and promote inhibition may also lead to release of vasoactive substances onto nearby vasculature. GABA may also influence nearby *astrocytic* control of blood flow. Doengi *et al.* showed that GABA release triggered Ca^{2+} transients in astrocytes, which in turn led to constriction of the vasculature in olfactory bulb astrocytes of rats [227]. However, astrocytic $[Ca^{2+}]_i$ can exert bidirectional effects on vasculature (as discussed above) depending on local conditions.

3.2.4 Baseline GABA levels linked to BOLD responses

At the level of the whole brain, the link between GABA and BOLD responses has been assessed *in vivo* using a combination of MRS and fMRI studies. The local GABA concentration, as measured using ^1H -MRS, is a combination of vesicular, cytoplasmic and extracellular pools of GABA predominantly reflecting synaptic inhibition, metabolism and tonic modulation respectively [228]. Although MRS cannot distinguish between these pools, baseline GABA levels do reflect a component of tonic inhibition. In a study by Chen *et al.*, endogenous GABA levels were raised in rat brain by blocking an enzyme that catabolises GABA, and were found to correlate with significantly reduced BOLD signal amplitude during forepaw stimulation [229]. This suggests that the *in vivo* inhibitory tone could modulate the strength of resultant BOLD responses.

Several human studies have explored the effect of excitation-inhibition state on the local CBF and BOLD response. Muthukumaraswamy *et al.* showed an inverse relationship between BOLD signal change and resting GABA levels in the brain [230]. Similarly, Donahue *et al.* found an inverse correlation between baseline GABA+² concentration, measured using a spectral-editing MEGA-PRESS sequence at 3 T, and BOLD signal change [232]. Surprisingly, the same study also found a positive correlation between CBF, measured using an arterial spin labeling sequence (ASL), and the GABA+ concentration. This finding was addressed by a subsequent, more methodologically rigorous, study [233], which found that GABA+ concentrations were inversely proportional to the CBF in that region, after taking into account differences in arterial arrival time, suggesting that inhibitory tone is related to perfusion *in vivo* [233]. Further evidence for the effect of endogenous GABA concentration on blood flow was found when comparing variability in the haemodynamic response function (HRF) of volunteers. In particular, more dispersive (wider) HRFs were found to correspond with increased GABA concentrations; differences in the functional state of the microvasculature were proposed to explain these observed differences in latency between HRFs [234]. Such correlational studies, however, struggle to add mechanistic insight, but do inform

²GABA+ is used to describe GABA concentration which contains coedited signal from macromolecular resonances [231]

about a clear relationship between CBF, PBR and baseline GABA levels in the human brain. Exactly how baseline inhibitory tone mediates blood flow *in vivo* and BOLD responses is still unclear.

Finally, these findings have been extended with novel work on the nature of NBRs using MRS. Northoff *et al.* found that resting GABA levels, measured using a 2-D J-resolved PRESS sequence, were predictive of NBR signal change in the anterior cingulate cortex (ACC) during emotional stimulation [235]. This result suggests that there is a direct association between GABA and the magnitude of the NBR, once again hinting that inhibitory tone may also modulate the NBR as well as PBR.

Further work is needed to shed light on the nature of negative BOLD responses. In particular, the use of *in vivo* functional MRS (fMRS), which has previously focussed on the PBR [130, 177, 180, 236], may yield insight into functional changes occurring during the NBR. Such an investigation may help elucidate the presence, if any, of increased inhibitory activity as well as revealing some of the metabolic consequences of sustaining negative BOLD signals.

3.2.5 Summary

In order to provide context for the work presented in this thesis, an overview of the literature surrounding the generation of BOLD contrast and its potential neurophysiological mechanisms has been described in the previous section. This comprehensive review has shown that while the current understanding of PBRs is far from established, it is vastly more detailed than that of NBRs. From discussions in the previous section, it is clear that the nature of PBR has been slowly unravelled through a number of studies of electrophysiological, haemodynamic, and crucially, neurochemical changes during BOLD responses. A picture has emerged of a response driven largely by excitatory synaptic activity and mediated by a coupling between neurons, astrocytes and control of local vasculature. When contrasted to PBR, the previous section has identified gaps in the study of NBR and its mechanisms. In fact, a review of the literature has shown that even the mechanism of the T_2^* -decrease in NBRs has been recently contested through the ‘blood-steal’ hypothesis. Only recently has a significant body of evidence

been presented that counters this argument, such as the occurrence of NBRs across different vascular territories and, most compellingly, the finding of a reduction in neuronal spiking in these regions. Current evidence revealed that NBRs may serve to shape perception and could reflect deactivation of specific brain regions.

Neurochemical changes, measured using functional MRS (fMRS), have contributed to the understanding of PBRs, through measurements of small Glu and Lac increases upon activation, for example. In particular, these fMRS findings have been suggested to be indicative of an increased oxidative metabolism in PBRs. However, there has not been a comparable study of such neurochemical changes during NBRs. If the NBR is correlated to increases in local inhibition, then metabolic changes associated with a greater degree of inhibition would be expected. The previous section briefly reviewed such metabolic requirements of inhibition and found that the energetic cost of inhibitory processes in the brain is unclear, although, across the brain it is probable that inhibitory processes consume less energy than excitation. Thus, detectable metabolic changes associated with NBR remain unclear. Through a review of GABAergic inhibition and its effect on vasocontrol, however, the previous section highlighted tentative links between GABA and local control of vasculature, potentially mediated by a blocking of the calcium transients ($[Ca^{2+}]_i$) in astrocytes. Additionally, evidence of inverse correlations between local endogenous GABA concentration and the strength of BOLD signal changes suggests a link between both inhibition and blood flow. If potential hypotheses surrounding the nature of NBRs, such as NBRs reflecting regions of neuronal deactivation, are to be adequately addressed, then a study using MRS may prove useful by providing insight into the metabolic state and transient neurochemical changes associated with NBR.

The motivation behind the work in this thesis is the development of MRS methods to probe markers of neuronal function and metabolism in the brain using robust localisation with semi-LASER. The measurement of neurochemical changes during BOLD responses is, therefore, an ideal neurophysiological response to examine in this thesis, since it requires methods sensitive to small changes in concentration. There is also a unique opportunity to study metabolite changes associated with

NBR and directly compare them to the PBR, through MRS measurement using a two-voxel acquisition scheme. A direct comparison of simultaneous neurochemical changes associated with PBR and NBR is precisely the experimental aim of the work presented in Chapter 5.

3.3 The IDH-mutation and 2-HG

3.3.1 Glioma

Gliomas are tumours originating in the glial cells of the central nervous system. As such, gliomas can be either astrocytic, oligodendroglial, mixed oligoastrocytic or ependymal, according to the guidelines of the World Health Organisation (WHO) [237]. These guidelines also further stratify glioma into grades (I–IV), which are based on histopathological features such as mitotic activity, pleomorphism and necrosis, and loosely reflect tumour malignancy and patient prognosis [238]. The most common glioma is glioblastoma (or glioblastoma multiforme) accounting for 55% of all gliomas and having the lowest 5-year survival rate of only around 5% [238]. Glioblastomas (WHO Grade IV) mostly tend to develop *de novo* (primary glioblastoma), although a small number (15–20%) progress more slowly from a lower grade lesion (secondary glioblastoma) [238].

Insight from genetic studies

There has recently been a radical shift in the understanding of glioma progression (or gliomagenesis) away from conventional histopathological measures. Several high-throughput genetic analyses have revealed multiple molecular subtypes of the disease. The Cancer Genome Atlas (TCGA), for instance, is a large scale collaborative project that endeavours to characterise genomic alterations of several human cancers. In 2008, they published DNA analyses in a sample of 206, mostly primary, glioblastomas [239]. Their integrative approach revealed several genetic alterations, including the amplification of epidermal growth factor receptor (*EGFR*) and antagonists to tumour suppressor (*MDM2*), as well as mutations or deletions in several tumour suppressor

genes (e.g. *PTEN*, *TP53*, *NF1*, *CDKN2A*). Primary and secondary glioblastomas, however, progress differently and therefore acquire different genotypes [240]. In particular, secondary glioblastomas have a higher incidence of *TP53* mutation, which is known to be an early event in gliomagenesis and also found in many (~60%) diffuse low grade gliomas (WHO Grade II-III) [241]. Yet, this marker alone is not specific enough to differentiate primary and secondary glioblastomas.

3.3.2 Mutations in *IDH1* and *IDH2*

A large step in the understanding of the genetic basis of glioma has been the discovery of mutations in the genes encoding for isocitrate dehydrogenase (IDH) enzymes. By performing a large scale genome wide study (>20,000 individual genes), Parsons *et al.* found recurrent mutations in the *IDH1* gene in 12% of all cases [242]. When broken down into tumour type they found that the *IDH1* mutation occurred in the majority of secondary glioblastomas and very few primary glioblastomas. This finding was corroborated in 88% of secondary glioblastomas by Balss *et al.* [243]. Importantly, *IDH1* mutations have also been discovered in the majority of low-grade (WHO Grade II-III) gliomas with rates of around (70-80%) [243–245]. This finding hints at the importance IDH mutations play in gliomagenesis, especially given the fact that *IDH1* mutations appear to occur early on, before mutation in tumour suppressor *TP53* [245]. A fraction of low grade gliomas without *IDH1* mutations have corresponding mutations in the *IDH2* gene encoding for the mitochondrial IDH enzyme [244]. Mutations in *IDH2* are mutually exclusive to *IDH1* mutations and occur at a much lower frequency (31/1010 = ~3% of II–III diffuse gliomas [246]). Interestingly, mutations in *IDH1* have since been found in acute myeloid leukaemia (AML) [247] and many other tumour types [248].

IDH enzymes

Considerable scientific interest has been sparked since the discovery of IDH mutations, not least because of the important biological role IDH enzymes play in normal cellular metabolism. The discovery of IDH mutations has far-reaching implications for the wider study of cancer metabolism.

Isocitrate dehydrogenase enzymes come in three different types (IDH1, IDH2 and IDH3) and are split into two categories based on their dependence on either coenzyme NAD^+ (nicotinamide adenine dinucleotide) or NADP^+ (nicotinamide adenine dinucleotide phosphate). IDH1 and IDH2 are genetically very similar (~70%), are both NADP^+ dependent enzymes, and are structurally unrelated to the NAD^+ dependent IDH3 enzyme [249]. IDH1 resides in the cytosol and peroxisome, whereas IDH2 and IDH3 are found in the mitochondria. The function of IDH enzymes in healthy cells is the oxidative catalysis of isocitrate to α -ketoglutarate (α -KG) [250]. Figure 3.3 shows their cellular compartmentalisation and function. The IDH3 enzyme carries out its primary function in the citric acid (TCA) cycle, yet since mutations in IDH3 are not reported, the term ‘IDH enzymes’ will herein refer to only IDH1 and IDH2.

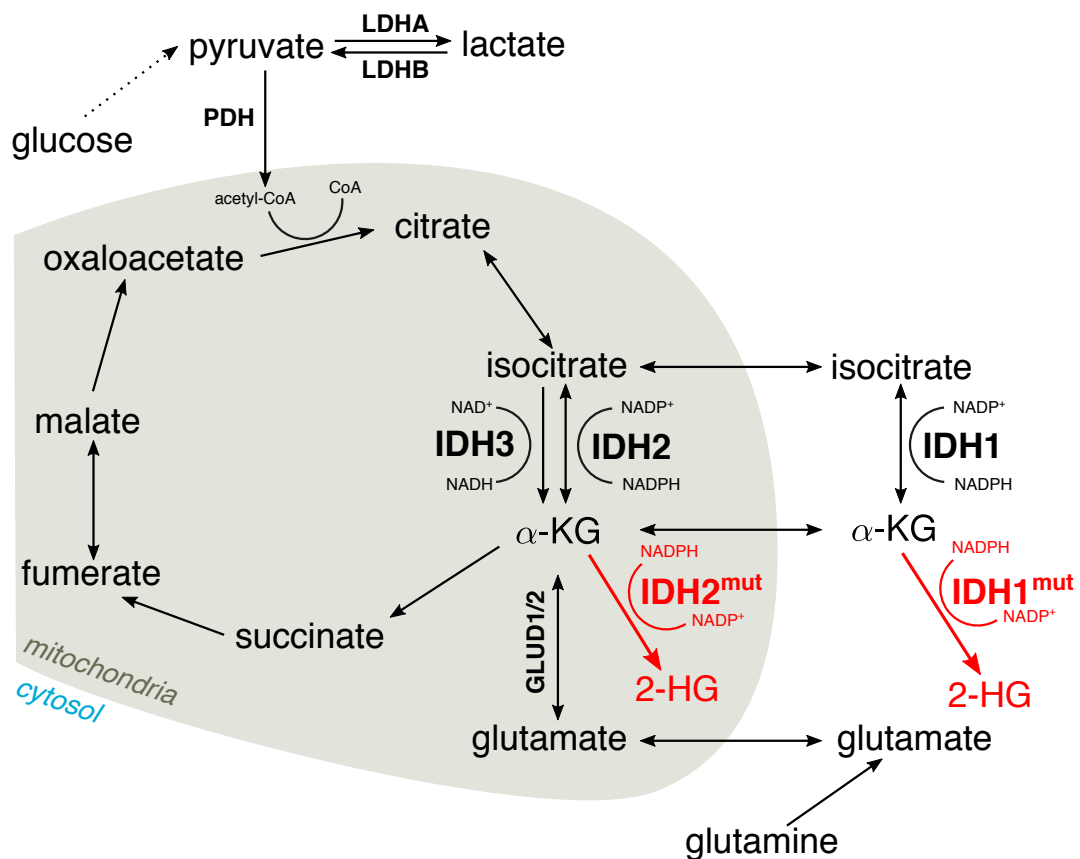


Figure 3.3: Schematic of the main metabolic pathways affected by mutated (red) IDH1 and IDH2 enzymes in the cytosol and mitochondria of glioma cells, which produce 2-HG. IDH enzymes sit at a crucial point in this metabolic flux through cells.

IDH enzymes have important functions in both cellular protection and energy

metabolism. Firstly, IDH enzymes are crucial for maintaining cellular defense against reactive oxygen species (ROS). The cofactor NADPH, arising from IDH catalysed α -KG production, is vital for the biosynthesis of many molecules [249]. Among them, is the reduction of the antioxidant glutathione (GSH) which protects against ROS damage [251, 252]. NADPH itself is also implicated directly in cellular redox control [249, 253]. Mailloux *et al.* have found that under oxidative stress, upregulation of IDH1 and IDH2 occurs, thereby producing α -KG and NADPH [254]. Secondly, IDH enzymes provide a source of α -KG — a substrate required for many further enzymatic reactions [255] — and they also regulate energy metabolism by controlling the reversible production of isocitrate and α -KG [256]. Further metabolic roles played by the IDH enzymes are fatty acid synthesis and glucose sensing [257].

3.3.3 Production of 2-hydroxyglutarate

A striking feature of IDH mutations is the consistency in type and frequency of mutation. For both *IDH1* and *IDH2*, the most common mutation arises from a single point substitution of guanine to adenine (G→A). This mutation has the consequence of replacing an arginine (R) residue with histidine (H) at codon 132 (R132H) [242]. Balss *et al.* found the occurrence of *IDH1* R132H mutations to be 92.4% and also found rarer mutations in the remaining cases of R132C, R132S, R132G, R132L and R132V, i.e. all replacing the arginine residue at 132 [243]. The rarer *IDH2* mutation is also characterised by a loss of arginine residue, in this case at position 172, and most commonly involves replacement with lysine (R172K) [244]. R172K has been reported to occur in 65% of *IDH2* mutations with the remainder being R172M (19%) and R172W (16%) [246]. Biochemically, these residues are vital for the functional behaviour of IDH-enzymes since they form the hydrophilic interaction which binds isocitrate [258]. Furthermore, these isocitrate binding sites are found to be highly evolutionary conserved across species [259]. The specificity of these mutations, led to the hypothesis that these mutations must confer a high selective advantage to tumour cells and also confer a potential gain in function [260, 261].

It was initially found that IDH-mutants have a reduced enzymatic ability to convert isocitrate to a α -KG [244]. This observation was supported by the fact that β -carboxyl bonds with isocitrate are disrupted in the mutated form of IDH leading to an observed 80% reduction in catalytic activity [262]. This leads to a decreased affinity for isocitrate and conversely increased for NADPH [263]. In 2009, Dang *et al.* showed that mutations in *IDH1* are neomorphic, thus conferring a gain in function to the enzyme, enabling the reduction of α -KG to R(-)-2-hydroxyglutarate (2-HG) [264]. They observed accumulation of 2-HG, at concentrations around 100-fold greater than in the wild-type, of around 5-35 μ mol/g. Given the functional equivalence of R132 in IDH1 and its counterpart R172 in IDH2, IDH2 mutations have, not surprisingly, also been shown to produce 2-HG and consume NADPH in the process [265].

Consequences of 2-HG production

Given the accumulation of 2-HG in IDH-mutated tumour cells it has been suggested that 2-HG itself acts as an 'oncometabolite' and as such has a direct influence on the path to gliomagenesis [266]. A comprehensive review of this is given by Waitkus *et al.* (2016) [267]. 2-HG has been suggested to be a competitive inhibitor of α -KG dependent enzymes and this is supported by the fact that the structures of (R-)-2-HG and α -KG are very similar. Evidence for this competitive inhibition has appeared in the form of 2-HG mediated inhibition of several α -KG dependent histone- and DNA demethylation pathways [268, 269]. The result of which is increased histone methylation (hypermethylation) and epigenetic alteration of the DNA, both observations that have been observed in tumours carrying an IDH mutation [270, 271]. The IDH mutation has also been shown to lead to a commonly observed, and highly hypermethylated, G-CIMP, glioma phenotype [272]. The IDH-mutation has also been shown to block the ability of cellular differentiation, a common hallmark of cancer, through 2-HG mediated competitive inhibition and subsequent hypermethylation [273, 274]. It is possible that 2-HG may be considered as an epigenetic modulator of cancer through the generation of aberrant DNA methylation.

Another key set of α -KG dependent enzymes are HIF-1 α hydroxylases. HIF-1 α (hypoxia-inducible factor 1- α) plays an important role in the regulation of cancer-related pathways including, glycolysis, angiogenesis and tumour growth, and their activity is modulated by several hydroxylases. There have, however, been mixed reports in the literature with respect to HIF-1 α activity in IDH mutations. In particular, levels have been shown to increase in mutated cells [262] explained by the inactivation of HIF-1 α hydroxylases with 2-HG. More recently, HIF-1 α levels have been shown to decrease in IDH-mutants through *promotion* of hydroxylases, specifically by (R-) enantiomer of 2-HG [275]. Whether 2-HG is a promoter or inhibitor of HIF-1 α activity remains to be clarified; several factors such as hypoxia, presence of other mutations and concentration of cellular 2-HG may contribute to these conflicting observations [267].

Metabolic Reprogramming of IDH mutants

Given the crucial role IDH enzymes play in cellular metabolism, in particular α -KG metabolism (3.3.2), the acquisition of an IDH-mutation places increased metabolic stress on the cell. How IDH-mutated cells respond to this challenge is an interesting question, one which may ultimately lead to the discovery of new metabolic markers or pharmacological targets for glioma. In particular, how do IDH-mutant cells maintain α -KG production to ensure the continuation of cellular processes? In their seminal work on IDH-mutations, Dang *et al.* found that concentrations of key TCA-metabolites such as, succinate, malate, fumarate and isocitrate were not significantly altered in IDH-mutants, thereby suggesting that these cells adjust their metabolism to maintain levels of these important metabolites [264].

It has long been known that a common hallmark of cancer cells is the heavy reliance on aerobic glycolysis (or conversion of glucose to lactate) – the Warburg effect [276, 277]. Analysis of gene expression, specifically of genes involved in the TCA-cycle and anaerobic glycolysis, has revealed key metabolic alterations in IDH-mutant tumours; namely the increased expression of glutamate dehydrogenase (GLUD) 1 and 2 in mitochondria [278, 279]. GLUD enzymes catalyze conversion of glutamate to α -KG and are hence critical for the glutaminolytic pathway. Chen *et al.* (2014) revealed that

upon genetic silencing of GLUD1/GLUD2 enzymes, tumour growth was hindered and that the introduction of GLUD2 enzyme in murine cells could rescue metabolic flux through TCA cycle in IDH1-mutants as measured by ^{14}C labelling [279]. The authors further implied that IDH1-mutant tumours could be taking advantage of the significant glutamine metabolism already present in the human cortex. It has further been shown that a large portion of the 2-HG in IDH-mutants is derived from glutamine (~70–80%) [280–282] and that blocking the action of glutaminase (an enzyme catalysing glutamine to glutamate) lowered α -KG levels and also reduced cell growth [283]. These findings suggest that IDH-mutants may have a metabolic preference for glutaminolysis over the conventional Warburg type glycolytic behaviour. Previous metabolomic analysis of IDH-mutants has supported this claim, with the finding of lower levels of glutamate and glutamine in IDH1 mutated tumours compared to wildtype as well as a reduction in overall viability of these cells grown in a glutamine free medium [284].

More recently, attention has turned to the dysregulation of other critical enzymes in IDH-mutated tumours, namely lactate dehydrogenase (LDHA/LDHB), critical for the aerobic glycolytic pathway. Both LDH enzymes (A and B) can catalyse the conversion of pyruvate into lactate in a reversible manner, however LDHB is thought to have a preference for the conversion of lactate into pyruvate [285]. It has been found that IDH-mutants tend to express LDHB more than healthy controls, whereas LDHA is overexpressed in IDH-wildtype tumours [278]. This discrepancy in expression reflects increased rate of aerobic glycolysis for IDH-wild type tumours - the frequently observed Warburg effect. Chesnelong *et al.* (2014) reported silencing of the LDHA enzyme in IDH-mutants, potentially arising from 2-HG induced hypermethylation, suggesting downregulation of the glycolytic pathway in these cancers [286]. Such downregulation is surprising because LDHA is commonly observed to be upregulated in cancer [287]. Interestingly, further downstream in the glycolysis pathway, Izquierdo-Garcia *et al.* (2015) have found a reduction in pyruvate dehydrogenase (PDH) activity, limiting the amount of pyruvate that enters the TCA cycle and is ultimately converted to glutamate [281].

3.3.4 IDH, personalised medicine and differential diagnosis

Over the past few years, our understanding of the pathogenesis of glioma has been rapidly improved by the addition of molecular and genetic information. A recent study by the TCGA research network (2015), found three distinct molecular subtypes of low grade gliomas, which were more predictive of clinical outcome than traditional classification [288]. These subtypes were characterised by deletion of the chromosome arms 1p/19q, mutation in TP53 and mutation in IDH. Molecular profiles of glioma are now included in the newest WHO guidelines on classification, reflecting the recent shift in diagnostic importance [289]. It is, therefore, clear that the diagnosis of glioma is moving away from a one-size-fits-all approach and becoming more reflective of these individual molecular differences. One of the major benefits of this change, is that treatments could be targeted to take advantage of particular metabolic alterations.

IDH1 and IDH2 as therapeutic targets

Conventional treatment of glioma has been a combination of chemotherapy and radiotherapy. Interestingly, Bleeker *et al.* have suggested that the reduced ability to produce the antioxidant NADPH, in IDH mutants, could lead to increased sensitivity to the ionising effects of radiotherapy and may explain the increased survival rate reported in IDH-mutants compared to wildtype [290]. IDH-mutants have also been shown to be a positive indicator of chemotherapy success using the standard glioblastoma treatment temozolomide [291].

One target, in the development of more personalised therapies, has been the selective inhibition of the mutated IDH1 and IDH2 enzymes. By reducing 2-HG levels in the cell it is hoped that the many downstream effects of 2-HG accumulation can be reversed [292]. Rohle *et al.* developed a drug to actively target IDH1-R132H mutations and was able to restore expression of certain genes relating to cellular differentiation and also inhibit the growth of mutated cells [293]. Similarly, Wang *et al.* targeted the IDH2-R120Q mutation (observed in AML patients) and, through reduction in 2-HG levels, were able to reverse the block on cellular differentiation [294]. It is not yet clear whether blocking the IDH mutated enzymes may reverse the survival advantage

conferred by these mutations. Interestingly, a novel vaccine-based approach, which induces a selective anti-tumour immune response based on the presence of IDH1-R132H mutations, has also been developed [295].

Finally, tumour treatments targeting the uniquely altered metabolism of IDH-mutants have been proposed [278, 296]. Particularly, attacking the dependence of IDH-mutants on glutaminolysis [283]. In particular, inhibition of the glutaminase enzyme resulted in reduced growth of IDH-mutants compared to wildtype cells [297]. Furthermore, targeting the increase in oxidative TCA metabolism in IDH-mutants compared to wildtype, by reducing the availability of cofactor NAD^+ , was shown to have a cytotoxic effect [298]. It is clear that as the understanding of metabolic reprogramming of glioma cells improves, more pharmacological niches for glioma treatment will arise.

3.3.5 Summary

The previous section highlighted the importance of studying IDH-mutations, and specifically 2-HG, *in vivo* in glioma patients. To summarise, the acquisition of an IDH-mutation is an event which occurs in the majority of gliomas and secondary glioblastomas. Evidence suggests that this mutation occurs early on during gliomagenesis and, hence, may provide significant prognostic value. In particular, it has been shown that patients with IDH-mutated tumours tend to have an improved survival rate over the wild-type, which may be explained by a possible increased sensitivity to radiotherapy and chemotherapeutic agents.

A review of the current literature in the previous section highlighted that there are numerous metabolic implications of acquiring an IDH-mutation. Alterations in cellular metabolism may be useful for diagnosing individual glioma subtypes and also for targeting pharmacological therapies to individual tumours. The primary alteration associated with IDH-mutations is the production of 2-HG. The subsequent accumulation of 2-HG has been shown to be detectable using MRS and, thus, accurate *in vivo* measurement of this mutation-specific marker opens up the possibility of non-invasive diagnosis of molecular subtypes of glioma. A review of the literature

revealed several consequences of neomorphic 2-HG production in cancer cells including hypermethylation of the genome, thereby modulating DNA expression in these tumours. Perhaps most strikingly, current evidence in the literature suggests that IDH-mutated tumour cells undergo significant cellular reprogramming to maintain crucial metabolic fluxes. This alteration of cellular metabolism appears to involve a transition from conventional glycolytic energy production to one supported by glutaminolysis. For the work carried out in this thesis, these findings are important, since they suggest that chemical alterations in key MRS-visible metabolites (e.g. Glu, Gln, Lac) may be detectable in IDH-mutated gliomas, provided the sensitivity of the method is high enough. Robust measurement of several metabolites, along with 2-HG, may, therefore, open up the possibility of *in vivo* metabolic profiling of gliomas. Thus, the IDH-mutation and, by extension, 2-HG detection using MRS, are a primary focus for the methodological developments using semi-LASER presented in this thesis (Chapters 6 and 7).

Two-voxel Hadamard-encoded semi-LASER at 7T

In this chapter, a method for performing 7 T MRS of simultaneous metabolite change from two voxels, with reduced chemical shift displacement, is developed and tested. Hadamard pulse-pairs capable of dual-band excitation are generated and tested in simulations. These Hadamard pulses are then positioned in a semi-LASER localisation scheme and the chemical shift displacement of the method is calculated. Measurement of inter-voxel bleed between the dual-banded excitation slice-profiles, as well as the applicability of the technique to measure concentrations of Lac and GABA at 7 T, is performed in a phantom. The technique is then applied *in vivo* in the occipital lobe of four volunteers and the neurochemical profiles arising from both voxels are compared. The technique presented here is then ultimately applied to the measurement of neurochemical changes during positive and negative BOLD responses in Ch. 5.

4.1 Introduction

Ultra-high field (≥ 7 T) MRS investigations are becoming more widespread in neuroscience and psychiatric research. Ultra-high field strength presents significant advantages in terms of the increased SNR and spectral dispersion [299]. While much of the multi-voxel MRS imaging (MRSI) development at these field strengths has focused on chemical shift imaging (CSI) [19, 300], the method suffers from a considerable point-spread function [96] and long scan times when water referencing is used. Hadamard encoding is an alternative that is theoretically able to fully leverage the benefits of increased SNR and spectral resolution seen using single voxel MRS [29, 301], while additionally acquiring signal from multiple regions simultaneously [80]. Hadamard encoding has been demonstrated extensively at 3 T [95, 98, 100, 302], and is suited to

acquisition over a small number of volumes and can be implemented in conjunction with CSI [303]. As RF power requirements increase almost linearly with magnetic field [57], RF-based localisation, at ultra-high field strengths, suffers from reduced available bandwidths, since pulse durations must increase to ensure the peak B_1 field of the pulse is below the maximum allowed value. Lower pulse bandwidths lead to diminishing gradient strengths and increased chemical shift displacement. Furthermore, the inhomogeneity of B_1 field at 7 T hampers accurate multi-voxel localisation over large regions. For Hadamard-encoding, these problems manifest as slice profile imperfections and leakage of signal between voxels or ‘voxel-bleed’, owing to the use of multi-frequency RF localisation (albeit smaller than CSI approaches [97]). Multiplexing acquisition methods such as pulse cascading have previously been proposed to reduce B_1 power, and also lead to improved slice-profiles and chemical shift displacement. However, these techniques can introduce differential T_2 -weighting on adjacent slices dependent on their order of encoding in addition to being only possible along one-dimension. A further issue is the shorter T_2 relaxation times of metabolites observed at ultra-high field strengths [73, 304], which places a constraint on the echo time (TE) of MRS sequences.

The use of adiabatic localisation is particularly suited to address many of the issues faced when using RF-based encoding at ultra-high field. Adiabatic localisation is insensitive to B_1 inhomogeneity and, because of the use of broadband frequency-swept refocusing pulses, suffers from considerably less chemical shift displacement than conventional localisation [20]. In particular, semi-LASER, is ideally suited to acquiring spectra with minimised T_2 evolution and in a shorter TE than full adiabatic localisation, which requires three pairs of refocussing pulses [18].

The use of a semi-LASER sequence for Hadamard encoding is, therefore, proposed in this work for the simultaneous acquisition of two voxels for minimisation of chemical shift displacement and improvement to the slice-profile. Transverse Hadamard encoding is a natural choice for two-voxel measurement because of the fact that n voxels can be obtained over n acquisitions, thus yielding optimal signal-to-noise ratio [80]. In the following work, the conventional 90° excitation pulse in the semi-LASER

sequence is replaced by a modified multi-banded asymmetric excitation pulse. The use of a previously optimised asymmetric RF excitation, is to allow for shorter TEs [305]. Properties of the Hadamard-modified excitation pulses, in terms of slice imperfection and voxel-bleed, are tested in a phantom. Finally, the method is demonstrated *in vivo*, in four human volunteers.

4.2 Aim

The aim of this study was to develop and examine a dual-voxel Hadamard encoded scheme suitable for simultaneous MRS acquisition at 7 T *in vivo*. To that end, several candidate dual-banded excitation pulses of different frequency separations were generated and characterised. It is intended that the method developed here is applied to the study of small neurochemical changes in a functional MRS study (Ch. 5). For the scope of this thesis, therefore, a single Hadamard pulse pair, based on its slice overlap and separation, was examined further. The chemical shift displacement, slice profile imperfections and voxel-bleed of the asymmetric excitation pulse were characterised in simulations and in a phantom. The sensitivity of the transverse-encoded semi-LASER Hadamard localisation method, to acquire reproducible spectra in two-voxels at 7 T, was then examined in four volunteers.

4.3 Dual-banded Hadamard RF pulse design

The development of the dual-banded excitation pulse began with modification to an already existing asymmetric pulse. The P10 pulse, shown in Fig. 4.1 (top left), is an asymmetric pulse with maximum B_1 of 13.5 μT and a pulse duration of 3 ms. Asymmetric pulses can be generated by truncation of existing sinc pulses to reduce the inter-pulse delay times, which arise from refocusing gradient lobes [306]. As a result, the TE of the sequence can be shortened to benefit spectroscopic acquisition. However, the truncation of slice selective pulses comes at the cost of a worsened slice selection profile. Starčuk et al. have generated asymmetric pulses with greater control over bandwidth and slice profile through combination of modulated Gaussian

functions [305, 307]. Such pulses can generate an improved excitation profile with fewer sidelobes, compared to a standard sinc excitation, as has been shown for the P10 pulse [308]. Thus, the P10 pulse was selected to be the excitation pulse for the modified two-voxel acquisition. The P10 pulse has an asymmetry factor equal to 0.78, measured as the proportion of the pulse that is taken up by the time from beginning to maximum intensity.

Five dual-banded versions of the P10 pulse were generated in the Matlab (Mathworks, Inc., Natick, MA) programming environment. These pulses were sampled ($N_{\text{sample}} = 256$) to maintain a duration of 3 ms while generating slice separations of $\Delta\omega = 3.0, 3.5, 4.0, 4.5$ and 5.0 kHz.

Two versions of these dual-banded pulses were constructed. One encoding anti-phased transverse magnetisation across a single band relative to the other in order to carry out Hadamard encoding (see Section 2.7.2). These modifications were performed using a single modulation of the original P10 pulse, $P_{\text{single}}(t)$. This modulation generated two separately encoded dual-banded pulses $P_{1,1}$ and $P_{1,-1}$ and is described by

$$\begin{aligned} P_{1,1}(t) &= -e^{i\pi\Delta\omega t} \cos(\pi\Delta\omega t) P_{\text{single}}(t), \\ P_{1,-1}(t) &= -ie^{i\pi\Delta\omega t} \sin(\pi\Delta\omega t) P_{\text{single}}(t), \end{aligned} \quad (4.1)$$

which can be shown to be equivalent to

$$\begin{aligned} P_{1,1}(t) &= P_{\text{single}}(t) + P_{\text{single}}(t) e^{i2\pi\Delta\omega t}, \\ P_{1,-1}(t) &= P_{\text{single}}(t) e^{i\pi} + P_{\text{single}}(t) e^{i2\pi\Delta\omega t}. \end{aligned} \quad (4.2)$$

It is clear from Eqn. 4.2 that the modulations described by Eqn. 4.1 lead to a superposition of two single-band pulses, $P_{\text{single}}(t)$, separated by a shift in frequency domain of $\Delta\omega$ (from the Fourier shift theorem, Eqn. 2.37). In addition, the second Hadamard encoded pulse, $P_{1,-1}(t)$, gains a π -phase shift across the original frequency band.

Simulations of the excitation profiles of these pulse pairs were then run using the *RF Pulse Tool* within the GAMMA/PyGAMMA simulation library of VeSPA (Versatile Simulation, Pulses and Analysis) [309]. The complex B_1 amplitudes and resultant frequency profiles of the generated pulses can be seen in Fig. 4.1. It can be seen that for the two smallest generated band separations, $\Delta\omega \leq 3.5$ kHz, there is a considerable degree of overlap of the two frequency bands. The multi-band pulses are all observed

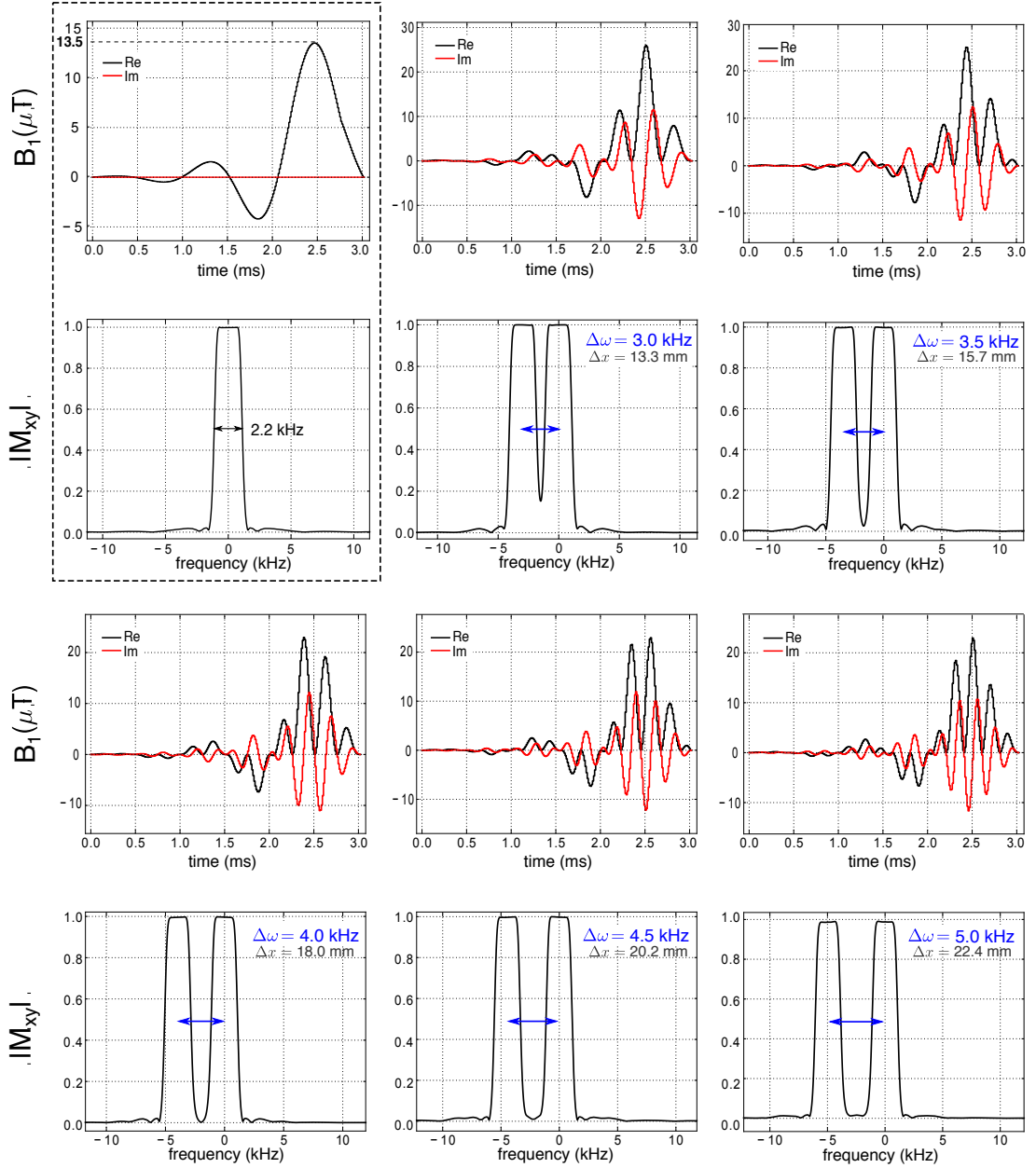


Figure 4.1: B_1 pulse profiles for the five dual-banded $P_{1,1}$ Hadamard encoded excitation pulses showing complex pulse shape (real *black* and imaginary *red* channels). Corresponding $P_{1,-1}$ pulses are not shown. The original single banded P10 asymmetric excitation pulse and frequency profile are also plotted (dashed box). For a pulse duration of 3 ms, the P10 pulse is measured to have a slice bandwidth of 2.2 kHz and maximum $B_1 = 13.5 \mu\text{T}$. Band separations for subsequent Hadamard pulses are given in blue and range from 3.0 to 5.0 kHz. Equivalent spatial separation of the slices at 7 T are given as Δx .

to acquire a complex B_1 component as can be expected from frequency shifting the excitation bands [87], which oscillate at a higher frequency for large $\Delta\omega$ separations. The separate bands of each pulse are observed to maintain the original slice bandwidth

per slice (2.2 kHz) for all separations. For separations $\Delta\omega \geq 4.0$ kHz, a maximum B_1 amplitude of $23\mu\text{T}$ was found to result in optimum slice excitation (i.e. $M_{xy} = 1$) across the whole slice bandwidth.

Since the generated excitation pulses are to be implemented in a slice selective scheme, the theoretical gradient strength required to localise a 10 mm slice at 7 T was calculated and found to be 5.24 mT/m. This is equated into a spatial separation, Δx , between each band shown in Fig. 4.1. Spatial separations ranged from 13.3 mm to 22.4 mm, for the smallest and largest frequency separations, respectively.

The $\Delta\omega = 4$ kHz pulse pair was selected to be examined further in both a phantom and *in vivo* owing to two criteria. Firstly, excitation pulses with separation $\Delta\omega \leq 3.5$ kHz were rejected on the basis of their high band-overlap and hence ‘voxel-bleed’. Secondly, spatial band separations $\Delta x \geq 20$ mm were considered too large for two voxels positioned across both hemispheres of the primary visual area (V1) in human.

The ability of the 4 kHz pulse to perform Hadamard encoding was tested in further custom-built simulations (to allow more control over simulation outputs) and can be seen in Fig. 4.2. The 4 kHz pulse pair of $P_{1,1}$ and $P_{1,-1}$ along with corresponding simulated transverse magnetisation profiles are shown. As is described by the encoding steps in Eqn. 4.2, the on-resonance frequency band of $P_{1,-1}$ contains components M_x and M_y of transverse magnetisation in anti-phase to that of $P_{1,1}$ (Fig. 4.2(c and d)). After Hadamard decoding the two magnetisation profiles (Fig. 4.2(e and f)), the signal from either voxel is clearly separated with negligible residual signal from the adjacent voxel.

4.4 semi-LASER implementation

The semi-LASER sequence was selected as the MRS localisation scheme for one-dimensional Hadamard encoding at 7 T because of its large insensitivity to B_1 and chemical shift displacement across the refocussing directions. The refocusing pulses were adiabatic full passage hyperbolic secant (HS4 R25) pulses (duration = 4.7 ms, bandwidth = 5.3 kHz, peak $B_1 = 28\mu\text{T}$) [20] (pulse profiles are shown in Appendix B). As a result, the net effect of chemical shift displacement across all three dimensions is minimised. The semi-LASER sequence implemented was symmetric in its positioning

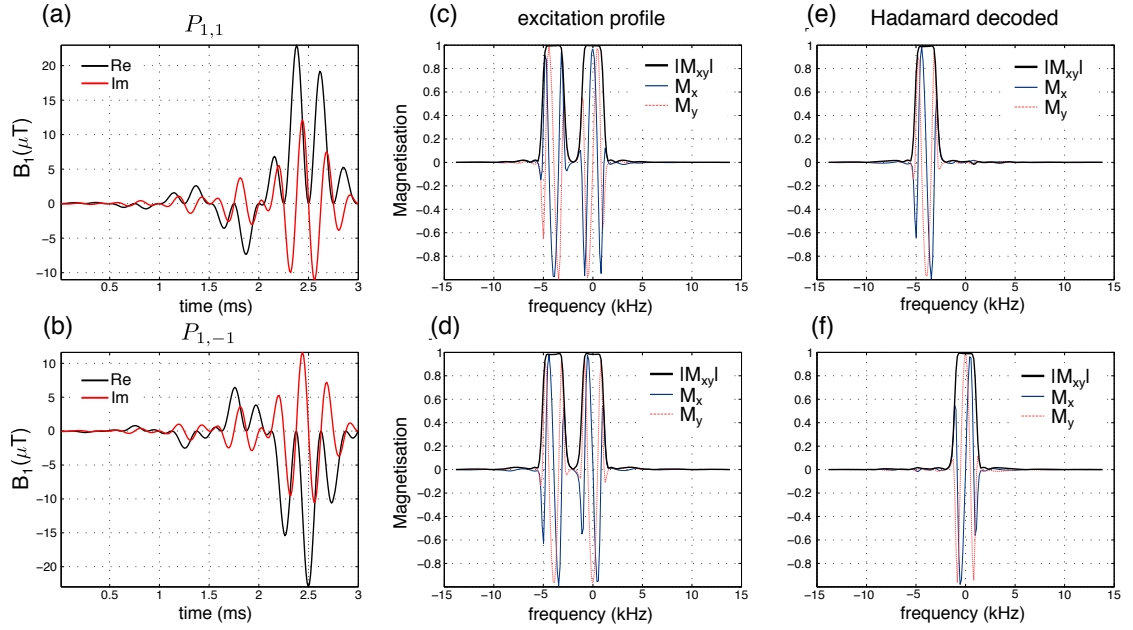


Figure 4.2: Simulated dual-banded 3 ms Hadamard-encoded pulses with 4 kHz band separation. (a,c): $P_{1,1}$ encoding pulse and corresponding excitation profile (showing M_x , M_y and $|M_{xy}|$ components of magnetisation). (b,d): $P_{1,-1}$ encoding pulse and excitation profile. Two single bands (e,f) are recovered after Hadamard decoding signals from (b,f)

of refocusing pulse pairs, i.e. the order of inversion was $180_z - 180_y - 180_y - 180_z$ [18]. The echo time was set to $TE = 32$ ms.

4.4.1 Outer Volume Saturation

In order to suppress any signal components arising from outside the prescribed VOI, outer volume saturation (OVS) bands were included, prior to localisation, in a manner similar to previous work at 4 T and 7 T [18, 301]. The outer volume saturation module is interleaved with VAPOR water suppression module and comprises of five pairs of slice selective broadband HS20 adiabatic pulses (duration = 5.12 ms, bandwidth = 8.03 kHz), which are thus insensitive to B_1 inhomogeneity. Three OVS pairs are positioned along the slice selective excitation direction x , along which chemical shift displacement is greatest, and two remaining pairs along y and z directions.

The positioning of the OVS bands needs to be adjusted to reflect the full-extent of the VOI. Therefore, the carrier frequency of one of the OVS_x pulse pairs was modified to take into account the additional spatial extent along x arising from multi-voxel

localisation. A spatial separation between the band edge and beginning of the outer-voxel suppression band of 7 mm was chosen as a compromise between effective outer volume suppression, and avoiding the suppression of any chemically shifted resonances from the voxel.

4.5 Calculation of Chemical Shift Displacement

The chemical shift displacement was then calculated for excitation and refocusing directions. The 3 ms dual-banded Hadamard pulses had a measured excitation bandwidth of 2.2 kHz at 7 T. For a voxel width of $V_x = 10$ mm, this pulse bandwidth corresponded to a slice selection gradient of

$$G_x = \frac{\Delta\omega}{\gamma V_x} = \frac{2.2 \times 10^3}{42.58 \times 10^6 \cdot 10 \times 10^{-3}} = 5.2 \times 10^{-3} \text{ [T/m]},$$

using Eq. 2.28.

The chemical shift displacement in this direction can, thus, be expressed in units of [ppm] as follows

$$\frac{\Delta x}{\Delta\delta} = \frac{\gamma B_0 \cdot 10^{-6}}{\gamma G_x} = \frac{7 \cdot 10^{-6}}{5.2 \times 10^{-3}} = 1.35 \text{ [mm/ppm]},$$

from Eq. 2.27 and Eq. 2.25.

For a chemical shift difference between the singlets of NAA (2.01 ppm) and Cr (3.03 ppm) of 1.02 ppm (= 303 Hz), this corresponds to a displacement of 1.38 mm or 13.8% of the voxel dimension.

Similarly for the refocusing directions, the HS4 adiabatic pulses have an inversion bandwidth of 5.3 kHz and for a 20 mm voxel width (later used in the *in vivo* study), this corresponds to slice selective gradient strengths of $G_y = G_z = 6.2$ mT/m at 7 T. The equivalent chemical shift displacement is therefore 0.56 mm/ppm. Between NAA and Cr, this is 0.57 mm or 2.9% of the voxel dimension.

In all three dimensions this shift between NAA and Cr results in two $10 \times 20 \times 20$ mm³ voxels which share a common volume of 81.4 % of the total voxel size.

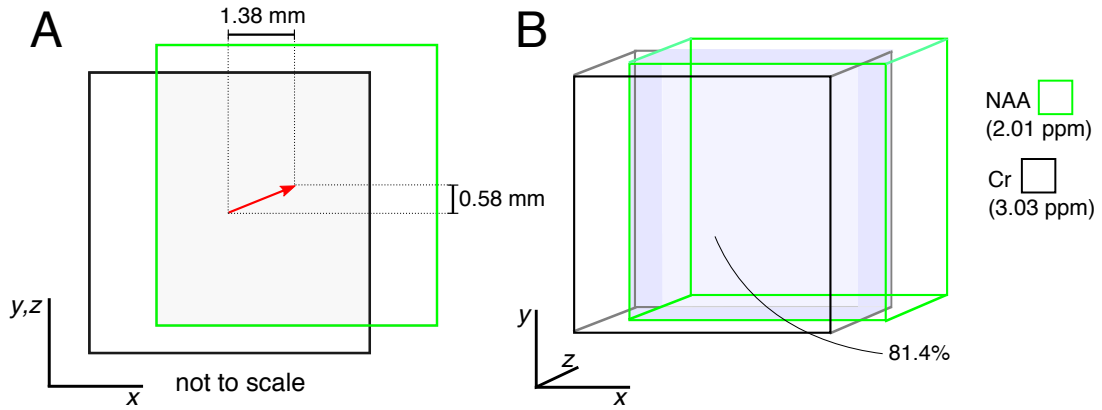


Figure 4.3: Schematic view of the chemical shift displacement in excitation (x) and refocussing directions (y, z) between NAA (green) and Cr (black) voxels. **A:** In two-dimensions, showing greater chemical shift across the excitation direction. **B:** Across three dimensions the total overlapping volume of both voxels is calculated to be 81%. Figures are not to scale and serve as demonstration only.

4.6 Slice Profile Measurements

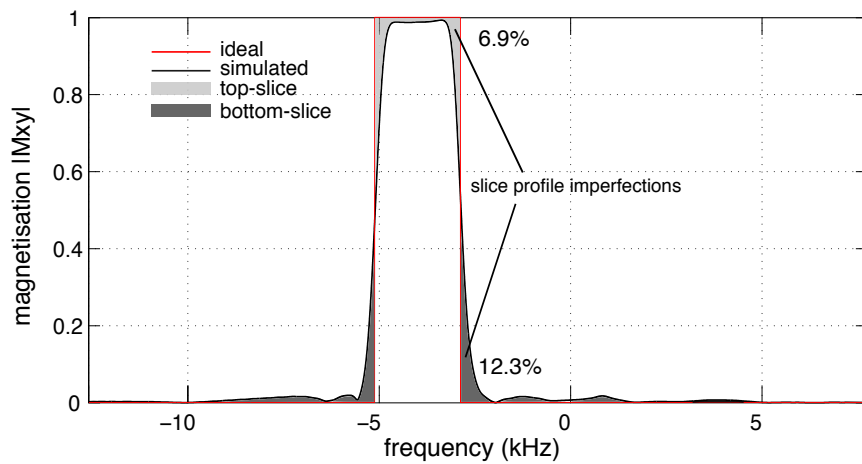


Figure 4.4: Calculation of slice profile imperfections for the simulated $|M_{xy}|$ profile for a single voxel, following localisation with the 4 kHz P10 Hadamard pulses. Simulated profile is shown (black) alongside the ideal case (red). Deviations as a proportion of the ideal profile are calculated and reported in figure revealing imperfections in the profile of 6.9% for the top of the profile and 12.3% below.

The performance of the above slice selective excitation pulses was then charac-

terised firstly in terms of (1) slice profile imperfections and (2) inter-voxel bleed. The former was evaluated through simulations of the transverse magnetisation behaviour following the pulses in comparison to an ideal excitation. Slice profile imperfections were measured for a single Hadamard encoded voxel and shown in Fig. 4.4. The deviation from the ideal case was measured as the fraction of signal (area integral) between the ideal and simulated profile and is split into two components. One is the fraction of the desired frequency band that is not fully excited (top-slice: shaded in light grey) and clearly leads to a loss in coverage. This fraction was calculated to be 6.9%. The second is the proportion of excitation that occurs outside of the desired frequency band (bottom-slice: dark grey). This imperfection generates overlap between neighbouring slices and hence contributes to the voxel bleed, which was calculated to be 12.3%.

The quality of the slice excitation profiles was then assessed in a spherical phantom containing oil. In particular, the proportion of signal that leaks into the neighbouring voxel (inter-voxel bleed) was examined. The phantom was scanned using a 7 T whole body MR system (Siemens, Erlangen) with a Nova Medical 32-channel receive array head-coil. The modified Hadamard encoded semi-LASER scheme was used with $TR = 8$ s and the voxel dimension in the Hadamard encoding direction was 10 mm.

Slice selection profiles along x were obtained by playing out a readout gradient, G_x , during acquisition, thereby frequency-encoding the excitation pattern along this dimension. This ‘one-dimensional’ image was obtained with a gradient strength $G_x = 0.503$ mT/m, resulting from a $FOV = 280$ mm and acquisition bandwidth = 6 kHz. The number of acquired samples was $N = 2048$, thus the spatial resolution of the resultant profile is 0.14 mm. Fig. 4.5 shows these reconstructed profiles after applying the inverse Hadamard encoding step on the two (free induction decays) FIDs, and applying a Fourier transform to the output. Both localised slices can be seen in blue and orange. The signal intensity is normalised to the maximum obtained. Also overlaid in Fig. 4.5 are the ideal excitation profiles (gray rectangles).

A measurement of voxel-bleed was then performed using this measurement in phantom. The definition of voxel-bleed is not consistent across the literature. For instance, it can refer to the amount of chemically shifted metabolites in adjacent

voxels [98] or the total signal arising outside of a particular slice [99]. Therefore, motivated by the application of the two-voxel technique in this thesis, a new measure was developed. The proportion of contamination of the signal in one voxel from the adjacent voxel is of primary importance for performing spectroscopic measurements of small metabolite changes. Thus, 'inter-voxel bleed' in voxel i from j is defined for the remaining discussion as follows

$$\text{inter-voxel bleed in } i^{\text{th}} \text{ voxel} = \frac{\text{signal from voxel } j \text{ within voxel } i}{\text{total signal from voxel } i} \times 100\%. \quad (4.3)$$

Evidently, the inter-voxel bleed in voxel i will not practically reach 100%. However, Eqn. 4.3 reflects the amount of signal present in one voxel from its neighbour. In this respect it is similar to previously defined metrics for voxel-bleed or leakage across slices [99]. This inter-voxel bleed is shown in Fig. 4.5 as the highlighted areas under each slice profile. From this, the inter-voxel bleed is calculated to be $(3.2 \pm 1.0)\%$ and $(3.0 \pm 0.7)\%$ for either slice.

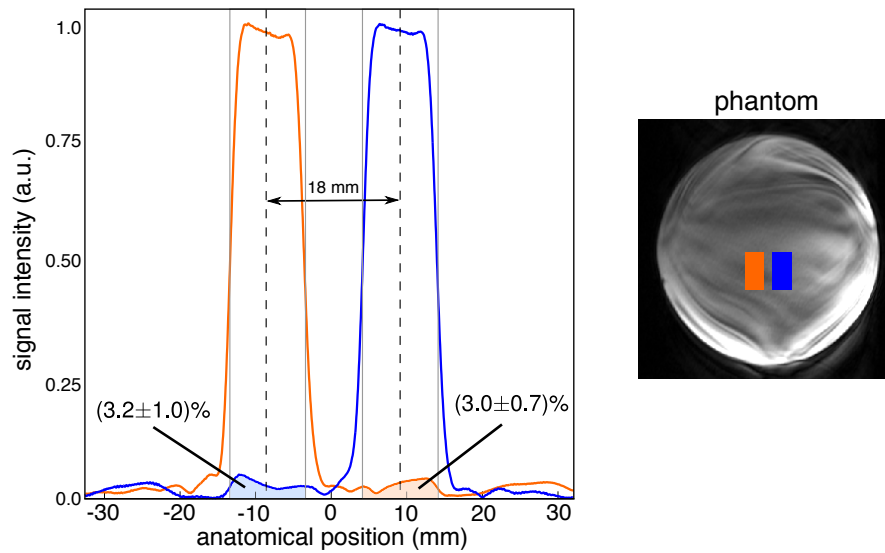


Figure 4.5: **Left:** Measured slice profile in phantom for Hadamard encoded, 3ms, 4 kHz separation pulse at 7 T. 2048 points sampled and spatial resolution was 0.14 mm. Inverse Hadamard encoding is applied to resulting data revealing signals from two voxels (blue and orange). The slice profiles are compared to ideal case (gray rectangles overlaid onto each slice). Voxel bleed is calculated as signal amplitude under each band (shaded areas) relative to ratio of total signal from the voxel. **Right:** Phantom containing oil overlaid with voxel placements for the two-voxel measurement.

4.7 Validation in phantoms

The ability of the sequence to perform robust MR spectroscopic acquisition from two voxels was tested in two further phantoms. Firstly, a phantom containing 10 mM lactate (Lac) and 10 mM acetate (Ace) was scanned. This was chosen because its spectral content contains a mixture of singlet (Ace) and multiplet (methine (CH) and methyl (CH₃) groups of Lac) resonances. Secondly, since the two-voxel method is intended to be used to measure small changes in neurotransmitter concentrations, namely γ -aminobutyric acid (GABA), a phantom containing pure GABA at the lower concentration of 1.5 mM was scanned.

The phantoms were scanned on a 7 T system (Siemens, Erlangen) with a Nova Medical 32-channel receive array head-coil. The dual-band semi-LASER sequence was used with TE = 32 ms, TR = 8 s and 180° bandwidth = 5.3 kHz. A total of 64 acquisitions were made in both phantoms corresponding to interleaving 64/2 = 32 times with each Hadamard pulse. Thus, 32 spectral averages per voxel were obtained. In the Lac phantom, the voxel dimensions were set to 10x20x20 mm³. In the GABA phantom, the total volume of the each voxel was halved to 10x20x10 mm³ = 2 mL in order that the two voxels could be both be positioned within the smaller confines of the phantom. Signals from individual channels were combined using an acquired unsuppressed water scan as reference. After Hadamard decoding is applied, the signals from each channel were phase-adjusted using the measured phase of the water signal and signal amplitudes were also weighted using this acquired water spectra, prior to combination. Coil combination was carried out using the Magnetic Resonance signal processing and analysis (MRspa) toolbox¹.

Unlike single voxel MRS, or multi-voxel with sequential acquisition [310], shim settings using multi-band RF localisation remain static and thus could not be optimised for each voxel separately. Therefore, in order to obtain an effective shim in both voxels, particularly across the Hadamard encoding direction, the shim volume encompassed the span of both voxels. This volume was shimmed firstly to second order with GRESHIM [311] and then first order shims were updated using FASTMAP [312].

¹<http://www.cmrr.umn.edu/downloads/mrspa/>

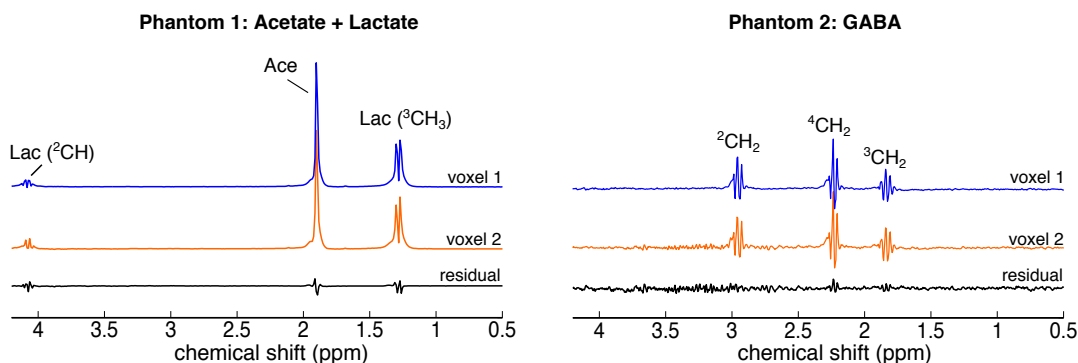


Figure 4.6: Spectra acquired using the two-voxel Hadamard semi-LASER sequence (orange and blue) in two different phantoms. The difference between the two voxel spectra is shown as a residual (black). **Left:** Phantom 1, containing Lac and Acetate with singlet resonance at 1.9 ppm. **Right:** Phantom 2, a custom-built GABA phantom, containing 1.5 mM. Spectra from both voxels are line-broadened post-acquisition by 2 Hz. A total of 16 transients per voxel were acquired from each phantom resulting in $32 \times TR$ (8 s) = 4 min 16 s acquisition time (TE = 32 ms). The voxel dimensions were 4 mL for Phantom 1 and 2 mL in Phantom 2.

Fig. 4.6 shows the spectra acquired from the phantoms containing lactate and GABA. Spectra were eddy-current corrected using an unsuppressed water reference, frequency and phase corrected and also spectrally aligned to known proton chemical shift values [313]. Linewidths were matched between voxels by manually broadening by 2 Hz through multiplication of the FIDs with an monoexponential function. Shown below the two voxel spectra is the difference spectrum (black) which is the residual signal between either voxel. The spectra clearly display near identical similarity in terms of resonance profile. In particular, the lactate multiplet at 4.09 ppm is detectable despite its position close to the water peak, although it is not readily detectable *in vivo* [313]. The second lactate multiplet at 1.31 ppm also has identical lineshape between voxels, indicating that chemical shift and J-evolution are similar in both voxels. The lineshape and signal intensity of the acetate singlet at 1.9 ppm is near identical in both voxels leading to a very low residual signal upon subtraction. The GABA spectra were acquired with a 50% smaller voxel size than lactate and thus possess half of the intrinsic signal amplitude. Yet, GABA remains detectable in phantom with 16 averages and low concentration (1.5 mM).

The spectral patterns of the individual resonances are identical between voxels

(Fig. 4.6). It is, however, apparent that the noise within the spectra of ‘voxel 2’ is larger than that of ‘voxel 1’ (Fig. 4.6 *right*) for the GABA phantom which is reflected in the larger residual signal. One possible explanation for this discrepancy may be the shim optimisation in this particular phantom. The GABA phantom was a long cylindrically shaped bottle and it proved hard to load coil sufficiently in order to achieve acceptable transmit voltages. Therefore, it was difficult to obtain a good shim solution across both voxels owing to a lack of sufficient B_1 power in this region. A sub-optimal shim solution within ‘voxel 2’ may have introduced a small amount of or spurious refocussing within this region, particularly around 3–4 ppm [314].

4.8 Validation *in vivo*

After validation in phantoms, the ability of the modified dual-band MRS sequence was then examined *in vivo* in four human volunteers. Volunteers were scanned on the same 7 T whole body MR system (Siemens, Erlangen) with a Nova Medical 32-channel receive array head-coil. Prior to carrying out MRS, a T_1 -weighted MPRAGE image with 1 mm isotropic resolution was acquired for voxel placement and later CSF correction. The semi-LASER sequence with modified Hadamard encoded asymmetric 90° dual-banded RF pulses (duration=3 ms; bandwidth = 2.2 kHz; peak B_1 = 23 μ T; band separation = 4 kHz / 18 mm) was used. For each volunteer, spectra were acquired from the occipital lobe using $10 \times 20 \times 20$ mm³ voxels positioned in either hemisphere (TR = 8s, TE = 32ms, NEX = 64, 180° bandwidth = 5.3 kHz) with VAPOR water suppression and OVS. Hadamard encoding was applied across the smallest voxel dimension. The inverse Hadamard step was then applied to yield FIDs from each voxel. Similar to the phantom study, FIDs were firstly eddy-current corrected, using an unsuppressed water reference, and then B_0 drift and phase corrected before being averaged (NT = 32). Metabolites were quantified with LCModel [315] using the unsuppressed water signal as reference. A basis set containing 19 metabolites was used to fit the data. Only those measured reliably (Cramér-Rao lower bounds (CRLB) $\leq 40\%$ and pair-wise correlation coefficients $r \geq -0.5$) from more than half of the volunteers are reported. Concentrations were corrected for the amount of CSF present in each VOI using the FSL segmentation

algorithm, FAST [316]. Concentrations were compared between the two voxels using paired two-tailed t-tests with a significance threshold set to $\alpha = 0.05$. Concentrations are reported relative to the total concentration of creatine (tCr) detected in each voxel.

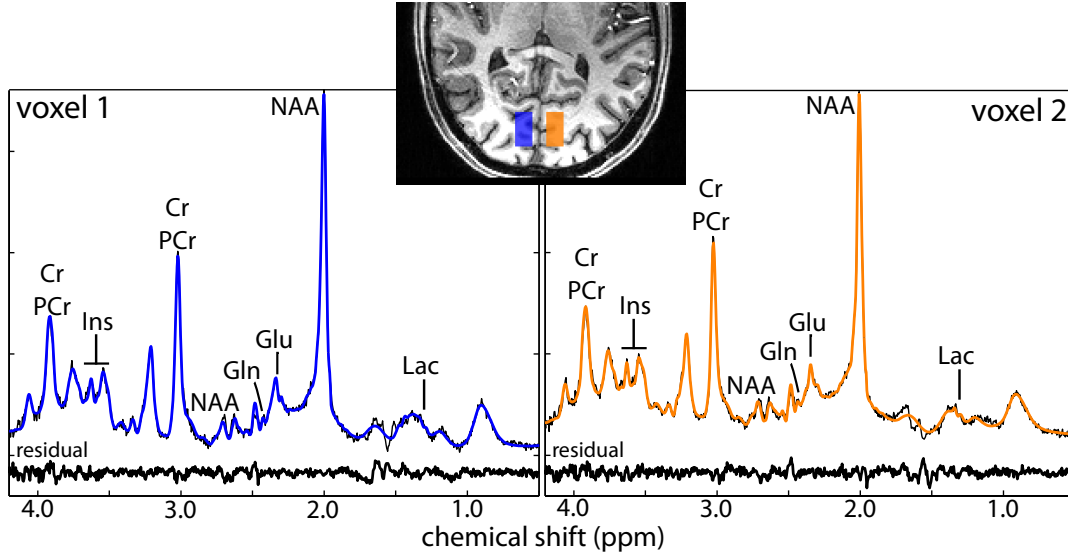


Figure 4.7: Spectra from the visual cortex of one volunteer (NT = 32). **Top:** T1 anatomical image with voxel positions overlaid. **Bottom:** The LCModel fits (blue and orange) from each voxel are plotted over the raw (phase and B_0 corrected) spectral data (black). The residual between the LCModel fit and raw spectrum is also shown underneath each spectrum. Some of the major peaks are labelled on the spectra for reference.

Fig. 4.7 shows an example of the spectral quality achieved with the two-voxel semi-LASER method from a single subject. The spectra are of comparable SNR and water suppression is of similar quality in both voxels. In addition, there is minimal contamination from outside the voxel from lipids, owing to the modified placement of OVS bands. Some of the major visible peaks are labelled in Fig. 4.7 and it is noted that Glu and Gln peaks are resolvable.

Alongside spectral acquisition, an unsuppressed water reference spectrum was also acquired. The linewidths of these water spectra were measured and averaged over all participants. These were calculated to be (14.0 ± 1.2) Hz and (14.8 ± 1.7) Hz for ‘voxel 1’ and ‘voxel 2’ respectively. Thus, there was no observable statistical difference between the linewidths in the two regions, which reflects the performance of comparable shimming across both voxels *in vivo* in the visual cortex.

The mean concentrations across all subjects of 14 detected metabolites are given in the graph shown in Fig.4.8. There is clearly close agreement between detected concentrations across all metabolites. In particular the method is able to resolve signals from Glu and Gln (with pair-wise correlation < 0.5) and other small concentration neurochemicals such as GABA and Lac with identical agreement between voxels. A small difference between myo-inositol (Ins) concentrations was detected between the two voxels ($p = 0.01$, paired t-test). However, this significance disappeared after carrying out a Bonferonni correction for multiple comparisons, $\alpha_{BF} = 0.05/14 = 0.004$.

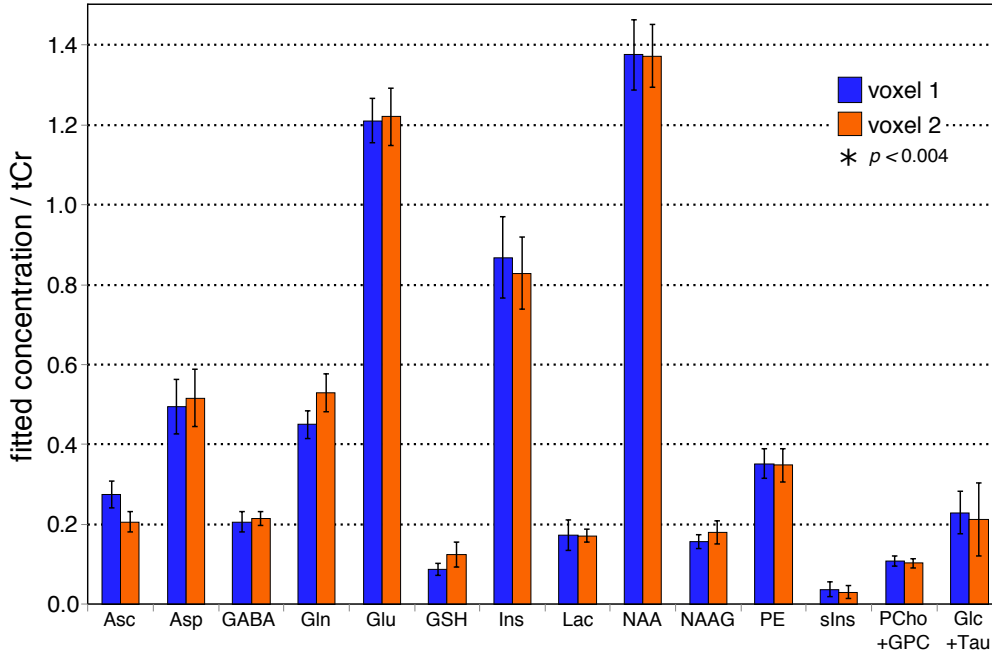


Figure 4.8: Average ^1H -MRS neurochemical concentrations *in vivo* from 4 mL voxels positioned in the occipital lobe of four volunteers. Error bars show one standard deviation and concentrations are reported relative to total creatine (tCr). Concentrations are obtained from LCModel fitting. Measured concentrations from both voxels (blue and orange) are shown to be in close agreement.

4.9 Discussion

The primary aim of this work was to produce a method for simultaneous two-voxel MRS at 7 T capable of sensitive neurochemical measurement with low voxel-bleed and chemical shift displacement. The modulation of an asymmetric RF excitation pulse, to achieve dual-banded excitation, was demonstrated through simulations, in phantoms

and *in vivo*. Hadamard encoding of these dual-banded pulses was able to produce high quality comparable spectra in the occipital lobe of four volunteers. In addition, chemical shift displacement was minimised in the two-refocussing dimensions, owing to the use of adiabatic inversion pulses. The signal bleed from the neighbouring voxel was measured to be low ($\sim 3\%$), although voxel separations were relatively large (~ 18 mm). Finally, it is proposed that such a method is ready to be implemented in a study of neurochemical changes during functional activity.

The benefit of using the P10 asymmetric pulse is that total TE of the sequence could be reduced to 32 ms, minimising T_2 losses compared to full adiabatic localisation. In this work, several dual-banded pulses were generated. However, pulses that generated separations with overlapping excitation bands ($\Delta\omega < 3.5$ kHz) (see Fig. 4.1) were rejected. For other applications, such as hybrid Hadamard and CSI, a closer slice separation and, hence, increased signal overlap, may be more tolerable. The P10 pulse is generated with constraints on excitation width as well as sidebands [305, 307]. The absence of large sidelobes (observed when using a truncated sinc excitation) is particularly important for dual-banded Hadamard encoded MRS, since these can result in significant inter-voxel contamination. It is, however, noted that some side-lobes are present in the single-slice profile of the P10 pulse [305]; these can be seen in Fig. 4.1 (top left). The sidelobes contribute to the inter-voxel bleed in the measured profiles in phantoms Fig. 4.5. The results revealed, however, that the amount of inter-voxel bleed was small ($3.2 \pm 1\%$ and $3.0 \pm 0.7\%$).

The measurement of voxel-bleed is problematic in multi-voxel acquisition since definitions vary depending on the application. For instance, Cohen *et al.* quantified bleed in terms of the amount of each chemically shifted metabolite signal in adjacent Hadamard slices [98]. Since voxels are separated by around 18 mm in the method presented here, chemical shift displacement of metabolites into the neighbouring voxel is avoided. The definition of inter-voxel-bleed in this work (Eqn. 4.3) therefore reflected the purpose of the developed method. The ratio of bleed is thus constrained by the amount of signal outside lying within the excitation bandwidth of the neighbouring voxel. If the definition of Goelman *et al.* [99] is taken, namely, the proportion of signal

outside the ideal rectangle to its area inside, then voxel-bleed is equivalent to the bottom-slice imperfection calculated in this work (Fig. 4.4) and is 12.8 %. This is lower than superposition of 5.12 ms sinc pulses (17 %) [97] yet does not match that achieved with fully multiplexed Hadamard cascaded pulses (4.9%) [99]. The definition of inter-voxel bleed in this work, therefore, should not be confused with the previous more conservative estimates of voxel-bleed.

A further aim for the development of the two-voxel method was reduction of the large chemical shift displacement experienced at 7 T. In line with this, the calculated chemical shift displacements of 1.35 mm/ppm in the excitation direction and 0.56 mm/ppm across refocussing directions for $10 \times 20 \times 20 \text{ mm}^3$ voxels was found to be low. These values correspond to 13.8% and 2.9% of the voxel dimensions. Chemical shift displacement arising from localisation with the dual-band P10 pulse is lower than using a superposition of sinc pulses (bandwidth = 1.7 kHz, duration = 5.12 ms), which have 0.88 mm/ppm chemical shift displacement for 5 mm slice widths or equivalently 17.5% of the voxel size [99]. A combination of cascading and multiplexing pulses has been shown to allow for greater gradient strengths and, hence, better chemical shift displacement at 7 T across a single dimensions of 0.50 mm/ppm[99]. However, this was not necessary in the current work, since the use of adiabatic refocussing pulses ensured that the total chemical shift displacement across all three dimensions is minimised. The minimisation of chemical shift displacement is highlighted by the large 81.4% overlap of shifted NAA and Cr voxels. The benefit of this low chemical shift displacement is the excellent spectral quality achieved in both voxels demonstrated in phantoms (Fig. 4.6) and then *in vivo* (Fig. 4.7). In particular, chemical shift displacement artefact for coupled spins is also minimised and, thus, reduces any sensitivity to chemical shift displacement artefact. Notably, spectral profiles were also free from lipid contamination.

Additionally, the use of adiabatic refocussing minimises the sequence's susceptibility to B_1 inhomogeneity, a particular problem at 7 T [57]. The B_1 -insensitivity of semi-LASER contributes to the spectral quality seen in Fig. 4.7 with only 32 averages owing to the robust refocussing of spins across the VOI, which is not dependent on

local B_1 variations [20]. Slice refocussing profiles along the y and z directions are, thus, less susceptible to this inhomogeneity.

The neurochemical profiles from either voxel in the occipital lobe of four volunteers show excellent agreement (Fig. 4.8). All metabolite concentrations were statistically similar and the standard deviations of metabolite measurements were small and comparable across voxels. There was a difference in Ins concentration between the two voxels, however this was not significant after applying a Bonferonni correction for multiple comparisons. The rejection of the null-hypothesis, i.e. that there is no difference between voxels, is increasingly likely when comparing over multiple metabolites. Incidentally, Ins may be more susceptible to a difference in detected concentration between voxels. Firstly, Ins has a triplet resonance positioned relatively downfield at 4.05 ppm close to the water resonance [313]. For this reason, fitting of Ins may be adversely affected by different water suppression in both voxels. If the efficiency of water suppression is poor in one voxel then the subsequent baseline may contribute to a higher estimated Ins concentration at this frequency. Secondly, this resonance also suffers from the most extreme chemical shift displacements, given its separation (1.35 ppm) from the RF carrier frequency at 2.7 ppm. In the slice direction, this corresponds to a displacement of 1.82 mm. In three dimensions this shift results in an Ins voxel with 77% overlap or, in other words, 23% of the signal from this resonance is obtained from outside the prescribed voxel location. For 'voxel 1' in Fig. 4.8, a lateral shift towards the mid-line of the brain may result in obtaining spectroscopic information from regions of different tissue content in both voxels.

The proposed two-voxel method would need to be modified in future work if encoding of more voxels in the excitation direction is required. It would not be feasible to superpose additional shifted pulses, since the peak B_1 of the dual-band pulses are already large at 23 μ T to maintain the 2.2 kHz voxel bandwidth. Further work in reducing B_1 requirements would also allow for a reduction in chemical shift displacement from greater allowable pulse bandwidths and may additionally improve the slice selection profile on the scanner [97]. Methods such as cascading the pulses in time have been shown to allow stronger gradient strengths for multi-banded pulses [98].

It is also suggested that a further practical step could be taken to allow generation of dual-banded RF pulses with tunable separation on the scanning environment. This development would increase the applicability of the two-voxel methods to other purposes and brain areas. The use of the technique in brain areas outside the visual cortex poses challenges to the Hadamard technique. In particular, both regions studied in this work were anatomically similar and thus possessed similar B_1 distribution (assuming no asymmetry in the left-right B_1 distribution in brain). Less symmetrically positioned VOIs may lead to voxels in regions of different susceptibility generating a higher demand on finding an optimum static shim for both regions. In fact, although one of the benefits of the Hadamard approach is the fact that slices do not need to be contiguous, large slice separations make optimising shims in both regions difficult. For this reason, sequential acquisition combined with dynamic shimming at 7 T may provide a better solution [310].

Finally, it is suggested that future work may investigate the use of the receive coil sensitivity profiles to help separate signals in a similar fashion to multi-band imaging in MRI with sensitivity encoding (SENSE) [317]. Unlike MRI, however, the necessity for large bandwidth pulses constrains minimum echo time and SAR, particularly for high order multi-band pulses. Boer *et al.* recently presented a simultaneous two-voxel method based on sensitivity profile encoding at 7 T, thus demonstrating that multi-band spectroscopy may be feasible [318]. However, it is envisaged that voxels with close separation, such as in the occipital lobe demonstrated in this work would prove challenging to separate with coil sensitivity alone. A combination of Hadamard encoding and SENSE reconstruction may provide a means to separate individual signals across multiple regions. However, the design of lower peak-power large bandwidth multi-band pulses for MRS is necessary.

The design of custom multi-banded excitation using specific constraints may also help reduce the amount of voxel-bleed. For instance, this could be achieved using a modified (Shinnar-Le-Roux) SLR algorithm [319]. The nature of the SLR algorithm means that side-lobes can be reduced considerably and instead the profile is limited by a degree of ripple in the pass- and rejection-bands [36, 320]. A combination of SLR and

the use of time varying gradients, as in the variable rate selective excitation (VERSE) technique [321], may also allow for the minimisation of RF pulse power requirements for such high bandwidth multi-band MRS pulses.

4.10 Conclusion

This work has shown that the use of dual-band Hadamard encoding in a semi-LASER sequence leads to high spectral quality and low chemical shift displacement at 7 T. Modification of the excitation pulse led to high spectral quality in two-voxels measured in the occipital lobe of five volunteers. Voxel bleed, arising from slice profile imperfections, was small compared to conventional methods. Furthermore, the chemical shift displacement of the localisation scheme was kept low in all dimensions (0.56 ppm / mm across the excitation direction) due to the use of broadband adiabatic refocusing. Future work may investigate methods to encode multiple spatial locations such as SENSE reconstruction, however the development of large bandwidth, low peak power, RF pulses is required. For the purposes of *in vivo* two-voxel measurement in the visual cortex, the current technique could readily be implemented for the study of simultaneous change in metabolite concentrations.

5

Neurochemical changes in positive and negative BOLD

In this chapter, a novel study of the metabolic changes associated with blood-oxygen-level-dependent (BOLD) responses is carried out in human volunteers. Using the two-voxel Hadamard method from Chapter 4, metabolic changes in negative BOLD responses, which may reflect areas of neuronal suppression, were measured *in vivo*. One of the aims of the study was to test the hypothesis that such regions may reflect transient increases in GABAergic activity as measured by MRS. In addition, the metabolic concentrations of other key metabolites were measured during a visual attentional suppression stimulus which evokes positive and negative BOLD responses in primary visual cortex. The effect of the enhanced T_2^* -decay arising from a reduced CBF flow in negative BOLD was also measured in the acquired spectra and compared to the positive response. Finally, time-courses of metabolic changes were produced and spectral differences upon functional activation analysed. This study contributes to the growing body of literature surrounding the nature of the negative BOLD response in humans.

5.1 Introduction

Establishing a causal link between BOLD responses and underlying neural activity has been of fundamental importance for the conclusions of neuroimaging studies. Alongside conventional BOLD responses, regions of sustained decreases in BOLD signal are also often observed. These negative BOLD signals are poorly understood relative to their positive counterparts, and yet may reflect regions of neuronal suppression or deactivation in the brain. Understanding the mechanism underlying negative BOLD responses is, therefore, of unique interest for neuroscience. Previous studies have used functional MR spectroscopy to probe metabolic changes in the BOLD response

in vivo during visual stimulation. In this work, metabolic changes in negative BOLD responses are measured simultaneously with positive responses, using a novel two-voxel Hadamard encoded MRS acquisition at 7 T.

BOLD imaging has transformed neuroscience research after its was first discovered over 25 years ago by Ogawa *et al.* [105] and then harnessed during functional tasks in humans [110–112]. BOLD contrast in functional MRI (fMRI) is generated by a reduction in T_2^* signal de-phasing following an increase in the fraction of oxygenated blood in active brain regions. From an imaging perspective, fMRI-BOLD reflects a mismatch between increased local cerebral blood flow (CBF) and the local metabolic rate of oxygen consumption ($CMRO_2$). Logothetis *et al.* have performed seminal studies in monkey brain investigating the link between BOLD signals and neural activity [149, 150]. In their studies increases in electrical activity measured by local field potentials, a marker of synaptic activity, are better predictors of BOLD responses than neuronal spiking [150]. BOLD imaging, therefore, may better reflect local synaptic processing rather than neuronal spiking *per se*. The energetics of excitatory synaptic activity also point to an increased metabolic demand. Glutamatergic activity, and hence neurotransmitter cycling, has been linked to increased rates of glucose oxidation in ^{13}C studies [15, 133]. Furthermore, glycolytic metabolism in neurons and oxidative metabolism in astrocytes may be coupled [131]. The link between synaptic activity and blood flow has been the study of much recent work. Glutamatergic uptake in astrocytes, for instance, has been shown to induce vasomodulatory CBF responses through intracellular calcium rises [157] (for a review see: [156]).

Several fMRI studies also describe regions of sustained decreases in BOLD imaging signal during visual stimulation [181–184], attentional tasks [185–188], somatosensory stimulation [189, 190] and motor stimulation [191–195]. However, compared to the positive BOLD response there has been relatively little research on its neurometabolic origins.

Negative BOLD responses (NBRs) reflect regions of reduced T_2^* from baseline and, hence, increase in deoxyhaemoglobin content. Since NBRs often occur adjacent to regions of conventional *positive* BOLD responses (PBRs), NBRs were initially suggested

to be regions of reduced CBF arising from a blood-steal to the PBR regions [182]. However, the observation of negative and positive BOLD responses, located in regions fed by different vascular networks, for example in visual [188] and motor areas [191, 193], suggested that NBRs do not simply arise from a diversion of blood flow. Shmuel *et al.* measured reductions in CMRO₂ in NBRs with a greater relative decrease in CBF, suggesting that they reflect areas of reduced metabolic activity from baseline met by a large decrease in CBF [183]. In further work, Shmuel *et al.* performed electrophysiological recordings in negative BOLD responses in monkey and found reductions in neural activity as measured by local field potentials and neuronal spiking [205]. Furthermore, Devor *et al.* showed that deactivated neurons surrounding functionally active regions are marked by vasoconstriction and reduced oxygenation [206]. Deactivation of neurons, through a suppressive mechanism, may therefore underlie negative BOLD responses as observed in functional imaging studies. Further evidence for this comes from neuroscience literature; negative BOLD amplitudes have been shown to shape perception [190] and suppress activity in unattended visual locations [210, 211]. Negative BOLD may, therefore, serve to improve stimulus perception through suppression or inhibition of certain brain areas.

Previous studies have used *in vivo* ¹H-MRS to probe the BOLD response. Through combining fMRI-stimuli with spectroscopic acquisition (functional MRS), changes in neurochemicals can be measured as a function of the stimulus. Mullins *et al.* initially detected increases in glutamate (Glu) concentration during a pain stimulus at 4 T [174]. This has been replicated in a handful of studies at 7 T where rises in Glu of around 5% have been detected during activation [130, 177, 180, 236] using visual stimulation. The same studies have also detected concomitant rises in lactate (Lac) concentration (around 20%) and decreases in glucose (around 15%). Such changes have previously been suggested to reflect an increased oxidative metabolism upon functional activation [128]. In addition to metabolic changes, studies have measured the BOLD effect using spectroscopy via decreases in the spectral linewidth associated with T_2^* -increases of around 0.5 Hz [175, 176].

γ -Aminobutyric acid (GABA) is the main inhibitory neurotransmitter in the brain. GABA-mediated inhibition has been shown to result in reductions in intracellular calcium rises in Purkinje neurons [225]. Further, Caesar *et al.* found reductions in neuronal activity and reduced CBF that were concomitant with local-field-potential increases using a GABA agonist [224]. Hence, it has been proposed that release of vasodilatory molecules is attenuated during GABAergic inhibition and may lead to the reduced CBF observed in negative BOLD [226]. In addition, tonic GABA concentration has previously been associated with the strength of negative BOLD responses in humans [235]. Since the BOLD response is reflective of local synaptic processing rather than neuronal output, it is thus hypothesised that negative BOLD regions may reflect underlying increases in GABAergic inhibition during functional activation.

5.2 Experimental Aim

The aim of this study was to test the hypothesis of an increased GABA response, as well as examine other neurometabolic changes, during periods of negative BOLD response with MRS. A visual stimulus, combined with an attentional suppression task, was used to generate adjacent negative and positive BOLD in the visual cortex of human volunteers. Using the novel two-voxel Hadamard encoded acquisition (proposed in Ch. 4), the aim of this study was to measure simultaneous metabolite changes in regions of positive and negative BOLD at 7 T. The study also aimed to measure the effect of T_2^* -induced linewidth changes from both response types on the spectra. Furthermore, support for the hypothesis of increased inhibition, in particular measured by transient increases in GABA concentration, in negative BOLD regions was investigated. Metabolic changes were also directly compared to those of the positive BOLD response.

5.3 Methods

5.3.1 Experimental Setup

Subjects

A total of thirteen healthy volunteers were recruited to participate in the study (mean age: 27 ± 3 , 5 male, 8 female). All volunteers gave informed consent for participation, which was approved by a local ethics committee under a technical development protocol.

Visual Stimulation

The visual stimulus used in this work was initially developed by Gouws *et al.* [1], who gave permission for use of the stimulus as well as providing the original code. The visual stimulus is written in *PsychoPy* [322]. Fig. 5.1A shows the visual stimulation paradigm. Volunteers were asked to fixate on a constant central red square while a laterelised peripheral grating was presented on either the left or right side of the visual field. The grating comprised of a 120° sector spanning $(4-8)^\circ$. The stimulus was divided equally horizontally, and top and bottom halves were overlaid with a high-contrast drift grating (square wave, frequency = 6 Hz, contrast = 100%, 0.08 cycles/ $^\circ$), which rotated in opposite directions. The direction of rotation (clockwise *vs.* anticlockwise) changed pseudo-randomly and volunteers were asked to count the total number of reversal changes over the whole paradigm.

The choice of this visual stimulus paradigm in this study was motivated by two main reasons. Firstly, BOLD response regions were localised to the primary visual cortex of either hemisphere. Localisation of the stimulus to either hemisphere of occipital lobe precludes the possibility that negative BOLD generation in this study arises from a pure haemodynamic origin (or blood-steal), since both regions are fed by different vascular networks [188]. In addition, unlike previous fMRI studies in which pure visual stimulation generated negative BOLD in unstimulated regions [183], the attentional suppression element of this paradigm (counting reversals of the peripheral stimulus) is directly hypothesised to relate to a neuronal suppressive mechanism and the generation of negative BOLD has been shown to be absent without this task [1].

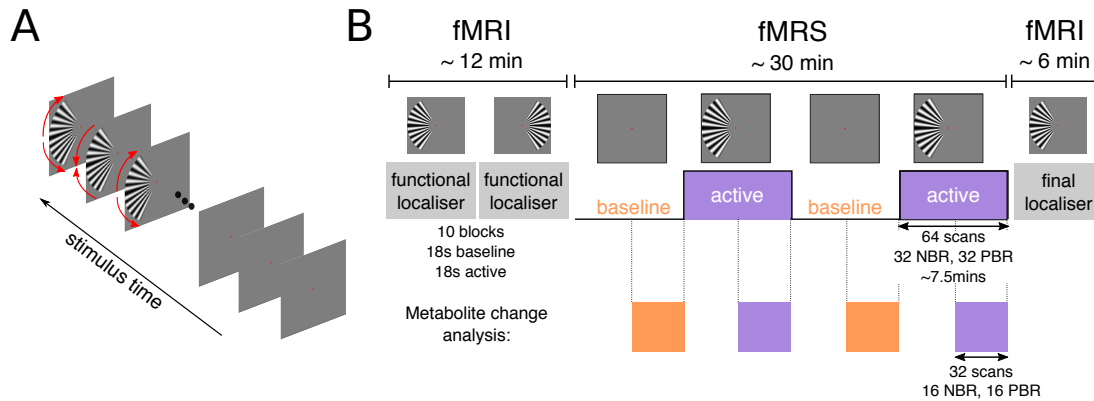


Figure 5.1: Experimental protocol for the fMRI-fMRS negative/positive BOLD study. **A:** The stimulus design together with attentional suppression task. Participants were asked to centrally fixate and count the number of reversals of a peripheral stimulus grating. **B:** The experimental outline. Two functional localisers are acquired to determine the optimum lateralisation of the stimulus in **A** for the fMRS acquisition. This is followed by a 30 min long acquisition of MRS, over two repetitions of the stimulus with block length of 7.5 mins (collecting 32 spectra for each voxel). Analysis of the data was performed over the latter half of each trial to avoid averaging over transient changes in metabolite concentration.

fMRI Protocol

Scanning was performed on a 7 T whole body MR system (Siemens, Erlangen) with 32-channel receive array head-coil (Nova Medical). For each volunteer, a 1 mm isotropic T_1 -weighted MPRAGE image was acquired (non-optimised, $TI = 1050$ ms, $TR = 1800$ ms, $TA = 2$ min). This was followed by two identical ‘fMRI localiser’ scans (see Fig. 5.1B) comprising of whole brain 2 mm isotropic EPI images ($TR = 3$ s, $TE = 25$ ms, $N = 120$, $TA = 6$ min). The localiser scans coincided with an fMRI block-design of the visual stimulation, presented over 10 trials of 6 scans (=18 s) baseline followed by 6 scans (=18 s) active. Lateralisation of the stimulus was changed between the two fMRI localisers (first left- then right-sided) in order to optimise MRS voxel placement. This was carried out to find PBR and NBR regions confined to the primary visual cortex (V1) of each hemisphere, given the variable positioning of the head in the coil and the altered field-of-view for each subject. BOLD data were analysed by means of general linear model (GLM) analysis on the scanner and responses were mapped relative to the T_1 -weighted image. A field-map image (B_0) was acquired for each subject (gradient-echo, $TE_1 = 4.08$ ms, $TE_2 = 5.1$ ms, $TA = 2$ min 2 s) for the analysis of fMRI data. Following this, the

two-voxel fMRS acquisition (details below) was performed during two lengthy blocks of the visual stimulation (448 s of baseline, 448 s active) with the chosen single-sided lateralisation. A final functional localiser was performed (post-fMRS), with identical acquisition parameters to those acquired pre-fMRS. Due to time constraints during a busy protocol, the post-fMRS localiser was only acquired in seven of the volunteers. This final fMRI acquisition was used to check if any significant adaptation had occurred during the lengthy fMRS section of the experimental protocol.

MR Spectroscopy Protocol

Spectra were acquired with the two-voxel Hadamard encoded semi-LASER sequence previously developed and proposed in Ch. 4. Acquisition parameters were as follows: TE = 36 ms, TR = 7 s, 180° bandwidth = 5.3 kHz, two-voxel separation = 18 mm. Water suppression was performed with VAPOR and outer volume suppression modules were included prior to localisation [301]. Based on the on-line GLM analysis, the neighbouring MRS voxels were positioned to maximise overlap with regions of maximum PBR and NBR in visual cortex, while minimising overlap with cranial lipids. A large region of (30×20×20) mm³, encompassing the combined span of both individual (10×20×20) mm³ voxels, was shimmed to second order with gradient-echo based shimming [311] and first order shims were updated with FASTMAP [312]. A total of 256 transients were collected over the entire duration corresponding to 64 for each trial (baseline or active) or 16 per voxel (Fig. 5.1). Finally, unsuppressed Hadamard encoded water spectra were acquired at the end of each fMRS experiment as internal references for the absolute concentration values. In one subject (subject 1), spectra were not obtained during the final activation block due to technical error. In addition, one further subject (subject 12) was presented with a reversed stimulation paradigm which began with active stimulation period followed by baseline due to experimenter error.

5.3.2 Analysis

BOLD Data

Pre- and post-fMRS BOLD maps, were generated using the FMRIB software library (FSL) [323]. Anatomical T_1 -weighted images were bias-field corrected and brain extracted. B_0 unwarping was performed on the EPI data using the fieldmap image. Motion was corrected using MCFLIRT and functional data were registered to each subject's anatomical T_1 -weighted image using the boundary-based registration algorithm [324]. Thresholding of functional activation maps was carried out using cluster-based analyses with a minimum z-statistic = 2.3. Spatial smoothing (FWHM = 5 mm) and a temporal high-pass filter of 36 ms was also applied to the data. A conventional GLM analysis was carried out, with the explanatory variable comprising of a gamma haemodynamic response function (HRF) convolved with the stimulus block design.

For the analysis of BOLD responses, an image mask from each $(10 \times 20 \times 20)$ mm³ MRS voxel was created and applied to the pre-fMRS functional data.

MR Spectroscopy

Absolute metabolite concentrations were corrected by the fraction of CSF in each voxel. Bias field corrected T_1 -weighted images were segmented into partial volume estimates using the FMRIB automated segmentation tool (FAST) [316]. MRS voxel locations were then registered from their native space to each T_1 -weighted image using the SPM8 toolbox¹. Masking the MRS voxels with the partial volume estimate yielded the fraction of CSF in each voxel.

The 256 acquired free induction decays (FIDs) were firstly Hadamard decoded into two datasets corresponding to PBR and NBR voxels. Each dataset was then eddy current corrected and coil-combined using the unsuppressed water reference signal with the Magnetic Resonance signal processing and analysis (MRspa) toolbox² for Matlab (Mathworks, Inc, Natick MA). PBR and NBR spectra were frequency aligned and phase corrected.

¹<http://www.fil.ion.ucl.ac.uk/spm/>

²<http://www.cmrr.umn.edu/downloads/mrspa/>

For the analysis of metabolite concentration changes, data from the initial half of each functional trial (baseline or active) were rejected to avoid bias from transient increases or decreases in metabolite concentrations (see Fig. 5.1). This is similar to the method employed in a previous study [180]. The remaining spectra were then averaged separately over the remaining parts of the active and baseline conditions to create two datasets for each voxel (PBR and NBR) containing $N = 32$ spectral averages.

To investigate individual metabolite timecourses, all spectra were included in the analysis and sliding window averaging was performed on the spectra from each subject. Spectra were averaged in sets of 16 transients to maintain reasonable SNR and a sliding offset of 8 transients was used, which resulted in a dataset of 14 timepoints at a temporal resolution of 112 s.

For the quantification of neurochemical concentrations in this study, LCModel was used to fit individual metabolites [315]. The basis set contained the following metabolites: alanine (Ala), ascorbate (Asc), aspartate (Asp), GABA, glucose (Glc), glutamine (Gln), glutamate (Glu), glutathione (GSH), *myo*-inositol (Ins), lactate (Lac), *scyllo*-inositol (Scyllo), taurine (Tau), phosphorylethanolamine (PE), phosphocholine (PCho), glycerolphosphorylcholine (GPC), creatine (Cr), phosphocreatine (PCr), N-acetylaspartate (NAA) and N-acetylaspartylglutamate (NAAG).

Only those metabolite concentrations with corresponding CRLBs $< 70\%$ were accepted. Statistical testing was performed using paired t-tests with a significance threshold of $\alpha = 0.05$ (two-tailed unless otherwise stated).

Finally, metabolite changes during functional activity were sought in the spectral data by means of a difference spectrum (see Fig. 5.2). Such an analysis has been performed in the context of PBR in many previous fMRS studies [130, 177, 180]. Averaged spectra were generated across all subjects for baseline and active periods using data from the latter half of each block ($n = 12$; subject 1 was excluded because of the absence of a final active stimulus condition). Spectral averaging across subjects was performed by minimising the residual of the NAA peak between individual spectra.

Each spectrum was ‘registered’ to a template subject free of contamination (PBR: subject 2, NBR: subject 10). This involved frequency-aligning each spectrum to the

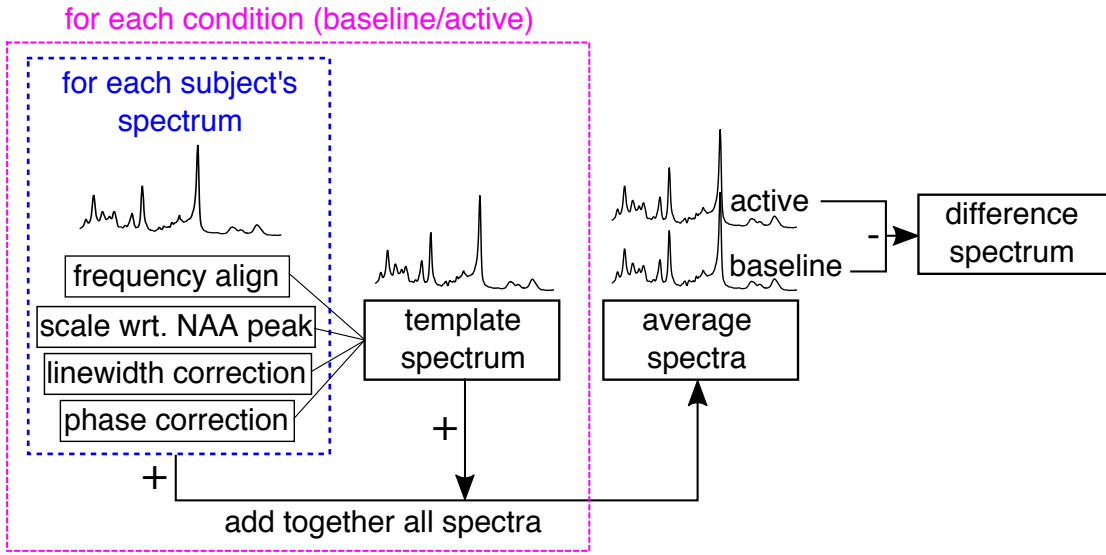


Figure 5.2: Outline of the post-processing pipeline for calculation of the difference spectra. A template spectrum was chosen from both PBR and NBR subject datasets to which each subject was registered.

template and scaling such that the amplitude of the NAA peak was matched. Spectra were subsequently either line-broadened or narrowed and small zero-order phase corrections were applied. Custom software was developed in Matlab (Mathworks, Inc. Natick, MA) to achieve this inter-subject averaging, which allowed visualisation of the residual signal after each spectral correction was applied (shown in Appendix D). Once each subject's spectra were matched to the template, an average was taken over all subjects resulting in a dataset made up of $12 \times 32 = 384$ individual transients for each condition.

5.3.3 LCModel fitting

LCModel performs an unparameterised fitting of a linear combination of prior basis sets on spectral data. Metabolite concentrations are then ascertained through calculation of the area under each fitted signal. In theory, LCModel should account for variability in spectral linewidth through the use of area integration. However, for very small BOLD-induced linewidth changes 0.4–0.5 Hz [176], changes in LCModel concentration outputs of the order of 1% have been suggested by Mangia *et al.*, who have used artificially broadened spectra LCModel fitting to simulate this effect [175].

Thus, a processing step involving *post-hoc* line-broadening of the spectra is necessary to ensure that small detected concentration changes are the result of other influences rather than a BOLD-induced line-narrowing artefact. Line-broadening of fMRS spectra has, therefore, been carried out by numerous groups investigating neurochemical changes during BOLD responses [130, 177, 180].

Water Correction

LCModel analysis scales the data by a factor, f , as described in the LCModel manual [315]. This factor is a ratio of the basis set signal intensity to the normalised unsuppressed water intensity W , acquired during the scan. The water scan is normalised by dividing the calculated signal area by the number of protons contributing the signal ($= 2$), the concentration, C , and a factor accounting for relaxation, R , as follows

$$W = \frac{\text{Area of Water Peak}}{2CR}.$$

In this work, the concentration of water was corrected for the fraction of CSF in the voxel f_{CSF} (described above and assumed to comprise of pure water) by applying a partial volume correction. The concentration of pure water ($\rho_{\text{water}} = 1000\text{g/L}$, molar mass = 18g/mol) is 55.555 M ($= 1000/18$). Thus, the total concentration of water in the voxel is then

$$C = 55.55 \times \frac{f_{\text{CSF}} + (1 - f_{\text{CSF}})w_{\text{tissue}}}{1 - f_{\text{CSF}}} \quad [\text{M}],$$

where w_{tissue} is the water content of the tissue (grey and white matter) and is estimated to be 0.82 [325]. T_2 -relaxation of the water signal is then accounted for in R , whereby $R = \exp(-\text{TE}/T_{2\text{water}})$.

From mM to $\mu\text{mol/g}$

Given the concentration estimates of metabolites output by LCModel are expressed in molar ($1\text{M} = 1\text{mol/L}$), concentrations can be converted to mol/g such that

$$\frac{\text{mol}}{\text{g}} = \frac{\text{mol}}{\text{L}} \times \frac{\text{L}}{\text{g}}.$$

Given the very low concentrations of metabolites compared to water in the solution (or content of each voxel), the density (in g/L) can be approximated to that of water, $\rho_{\text{water}} = 1000g/L$. Thus,

$$\begin{aligned}\frac{\text{mol}}{g} &= \frac{\text{mol}}{L} \times \frac{1}{\rho_{\text{water}}} \\ &= \frac{\text{mol}}{L} \times 10^{-3}.\end{aligned}$$

Therefore metabolite concentrations in mM are approximated to concentrations in $\mu\text{mol}/g$ in this work.

5.4 Results

5.4.1 BOLD Responses

Thirteen subjects underwent a combined visual stimulation and attentional suppression task, which has previously been shown to evoke positive and negative BOLD responses in adjacent regions of the primary visual cortex [1]. As expected from the stimulus design, across all subjects, positive and negative BOLD responses were generated in contralateral and ipsilateral visual cortex, respectively, relative to the lateralisation of the peripheral drift-grating stimulus. Selection of stimulus lateralisation for the fMRS task was based on how restricted each response type was to the visual area of either hemisphere (left-sided: 10 subjects, right-sided: 3 subjects).

Fig. 5.3 shows the BOLD responses for the functional localiser fMRI acquisition from three subjects. Subjects A and C received left-sided stimuli whereas B received a right-sided stimulus. Fig. 5.3A(i) shows the BOLD z-statistical map for one subject. Large positive activations are observed in the right (contralateral) primary visual cortex V1, but also in the middle temporal visual area (V5) both contra- and ipsilaterally to the stimulation. Adjacent to this, a weaker negative BOLD response is observed in ipsilateral V1. Measurements of the BOLD signal amplitude in each MRS voxel (white rectangles in Fig. 5.3) were also performed. Fig. 5.3A(ii) shows the BOLD signal timecourse (% signal change) within each MRS voxel (PBR *top* and NBR *right*) corresponding to the fMRI voxel with maximum z-statistic. Fig. 5.3A(iii) plots the

distribution of non-zero BOLD signal changes from all responding fMRI voxels within the larger PBR and NBR MRS voxels. The median and 90th percentile of these distributions are also plotted on each figure. Clearly, the median BOLD response in the PBR voxel is positively responding (1.6 ± 0.02)% in this subject while the opposite is true in the NBR voxel (-0.78 ± 0.01)%. However, there is a small contribution from the opposite BOLD response type ($\lesssim 10\%$) in either voxel.

Fig. 5.3B(i) shows similar activations in a subject who experienced right-sided visual stimulation with median BOLD amplitudes of (2.63 ± 0.03)% and (-0.43 ± 0.03)% in the PBR and NBR voxels respectively, yet this subject has less contamination in either MRS voxel ($< 10\%$) and a large fraction of almost zero signal change (Fig. 5.3B(iii)). Mean BOLD response maps generated from the left-sided stimulus across all subjects ($n = 10$) are given in Appendix C and show a similar pattern of activation/deactivation. The mean activations from the right-sided stimulus is not generated because of the small number of subjects.

At an individual subject level, the spatial location of each BOLD response type was observed to deviate from that expected in some cases. For example in Fig. 5.3C(i), a large negative BOLD response can be seen, which is not restricted to ipsilateral V1 but extends to contralateral V1. The masked BOLD response for the *PBR voxel* thus contained a median *negative* BOLD signal amplitude of (-1.05 ± 0.07)%.

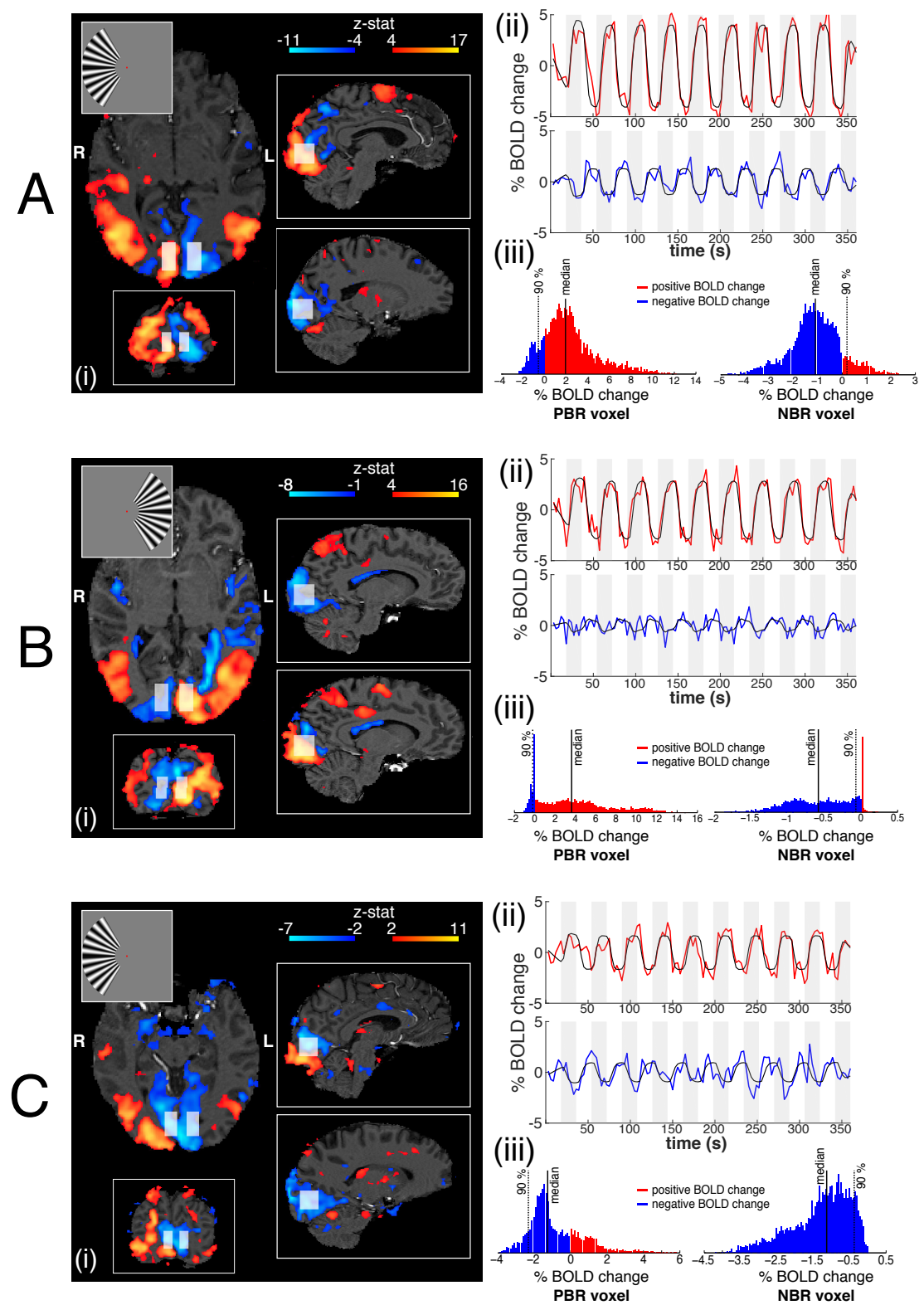


Figure 5.3

Figure 5.3: Summary of the response to the fMRI visual paradigm for three subjects (A), (B) and (C). Thresholded z-statistical maps (i) show significantly responding positive (red) and negative (blue) BOLD responses. Maps displayed on T1-weighted image and location of the MRS voxels is shown (white). The stimulus lateralisation is displayed in top left. (ii): BOLD signal timecourses (% signal change) corresponding to the maximum z-statistic in each MRS voxel (PBR: red, NBR: blue, PE: black). (iii): Histograms of the BOLD signal change in each MRS voxel, showing proportion of positively and negatively responding fMRI voxels ($N_{\text{bins}} = 100$, median (solid line) and 90th percentile (dotted line) also given). Subject C has a large proportion of negatively responding voxels within the PBR voxel

BOLD contamination of the MRS voxels

A degree of overlap from both responses within each MRS voxel may be expected, since the dimensions of the MRS voxels are of the same scale to the cluster size of the BOLD responses; spatial smoothing of the fMRI data may lead to more diffuse responses. Individual response maps are also expected to be highly subject specific. Contamination of each MRS voxel with opposite BOLD response types (i.e. negative BOLD in the PBR voxel), however, poses a significant problem for interpretation of the metabolic changes during functional activation. Therefore, a metric for rejection of data based on the level of BOLD contamination in each voxel was developed in order to remove severely contaminated datasets from subsequent analysis. A comparison based on percentage BOLD signal change within the voxel was not adequate since this varies across subjects. Therefore, thresholded maps of significantly responding fMRI voxels based on the BOLD z-statistics were used. A BOLD contamination metric for each MRS voxel, c , was therefore calculated based on the number of negatively and positively responding voxels, v_p , and v_n , respectively, whose z-stat > 2.3 (cluster-based threshold), such that

$$c_{p,n} = \frac{v_{n,p}}{v_p + v_n} \times 100. \quad (5.1)$$

Figure 5.4 shows the value of this contamination level c , for each voxel (PBR in red and NBR in blue), in each subject. It can clearly be seen that across all subjects the PBR voxels are more contaminated with negative BOLD response, $c_p = (27 \pm 18)\%$, (mean $\pm$ std. dev.) than the NBR voxels are with positive BOLD response, $c_n = (3 \pm 5)\%$. The largest contamination is observed in subject 5 (corresponding to Fig. 5.3C), containing

73% of significantly responding voxels arising from the negative response. A threshold based on this contamination was implemented, such that spectra arising from voxels which have contamination greater than one standard deviation from the mean level, were rejected. Spectral data from PBR voxels of subject 4 and 5 and NBR voxels of subject 3 and 7 were, therefore, removed from the analysis of metabolite changes.

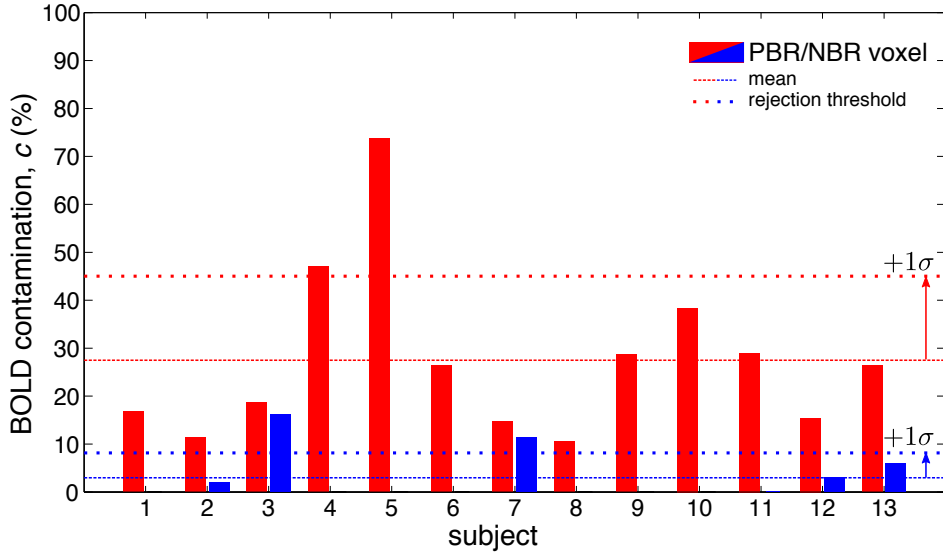


Figure 5.4: Amount of contamination in each spectroscopy voxel (PBR or NBR), arising from statistically significantly voxels of the opposite BOLD response, plotted for each subject. The mean (dashed) and standard deviation (dotted) of the contamination metric c from all subjects is plotted for each voxel type. Voxels with contamination $> \text{mean} + 1\sigma$ were rejected from further analysis.

5.4.2 Adaptation to stimulus

The BOLD data were then assessed for indications that adaptation to the stimulus occurred over the lengthy trial duration of the fMRS. Neural adaptation to repetitive stimuli can lead to reduced BOLD responses for subsequent repeat measurements using the same stimulus [326]. In particular, Fang *et al.* measured significant adaptation, and hence reduced BOLD responses, in primary visual area (V1) to stimuli after ‘long’ presentation (> 20 s) of an orientated grating stimulus [327]. Since each stimulus presentation during fMRS in this study was of the order of ~ 5 minutes, it was important to assess if any adaptation occurred during acquisition of fMRS data. Furthermore, it is not clear how negative BOLD responses may be affected by such

long stimulus presentation, although in this study, the randomly changing nature of the peripheral grating (similar to stimulus presented in [203]) combined with counting task was expected to minimise the effects of neural adaptation to the stimulus. Analysis of the BOLD data from the functional localiser scans taken pre- and post-fMRS was performed in seven subjects.

Fig. 5.5 shows a comparison of the BOLD response pre- and post-fMRS for a single subject (subject 6). Visual inspection of the thresholded z-statistical maps in Fig. 5.5A revealed similar activation/deactivation distributions between scans. Importantly, both PBR and NBR responses remained localised in V1. Interestingly, it was noted that the positive BOLD response appeared slightly reduced in coverage across the brain, yet the negative BOLD response had a larger spatial extent in the post-fMRS scan. The BOLD signal amplitude distributions of either voxel's fMRI responses revealed very similar distributions of BOLD signal change between timepoints (Fig. 5.5B), with a shift in the median BOLD response change in the NBR voxel towards more negative responses (-0.29 to -0.44)%.

Across all subjects the spatial maps of pre- and post-stimulus maps were similar. The mean, median and extrema of these BOLD response histograms were analysed for all subjects across the two scans. No significant difference in the mean ($p = 0.39$ and $p = 0.69$; NBR and PBR) or median BOLD response ($p = 0.46$ and $p = 0.88$; NBR and PBR) (see Fig. 5.6) was detected across the seven subjects. Interestingly, the only discernible difference was found in the maximum negative BOLD response in the NBR voxel, which was 17% *larger* than the initial pre-fMRS scan ($p = 0.029$, paired two-tailed t-test). Importantly for the conclusions of this study, the absence of a diminished BOLD magnitude of either response suggests that stimulus adaptation in V1 did not occur during the fMRS scan.

5.4.3 Spectral Quality

Fig. 5.7 shows an example of the spectral quality achieved with the two-voxel Hadamard technique in the study. Spectra arising from baseline and active periods are comprised of 32 spectral averages from the the final halves of each stimulation block (16 averages).

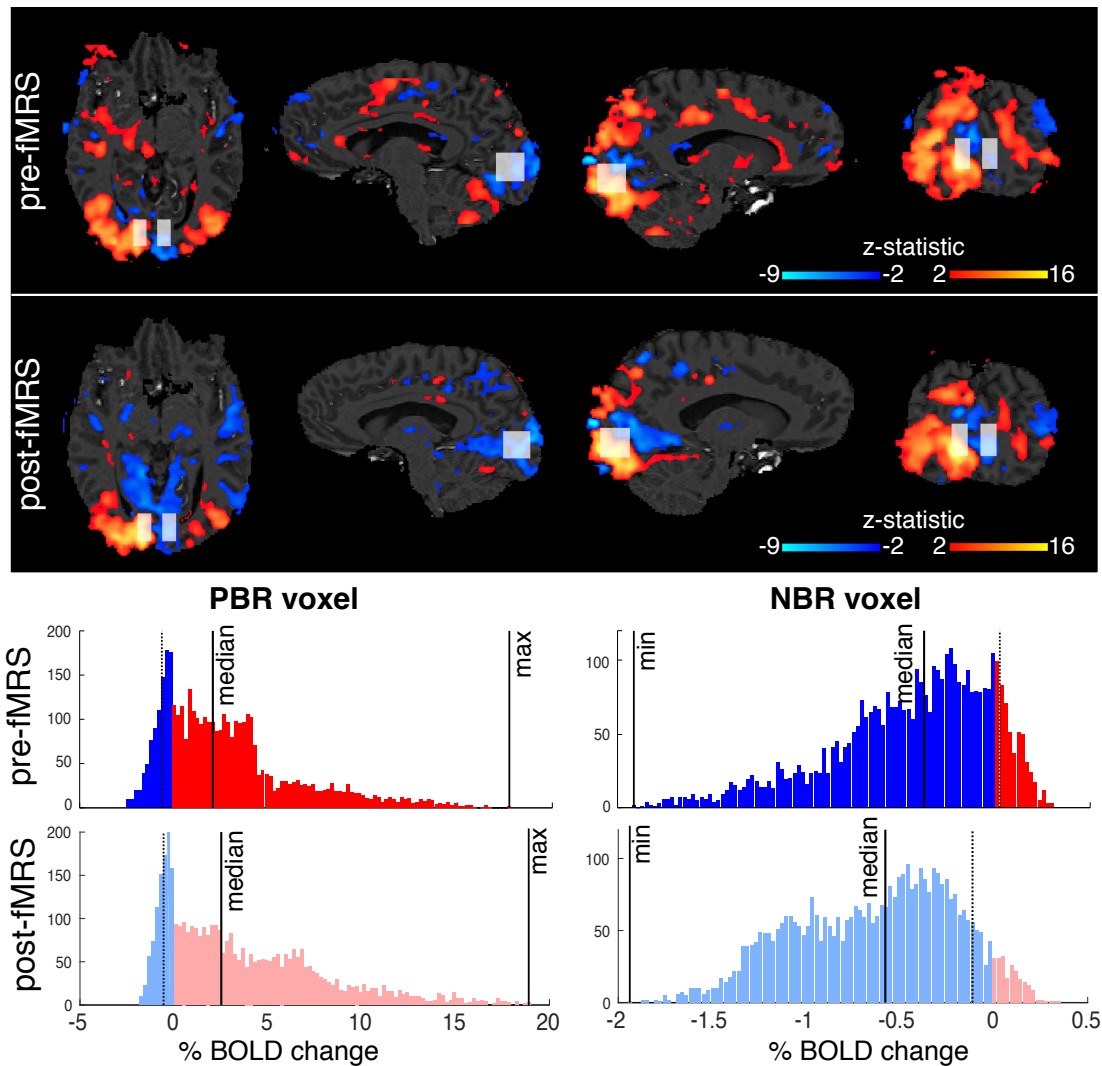


Figure 5.5: Comparison of functional activation acquired before (pre-) and after (post-) acquiring the 30 minute-long fMRS data, shown for a single subject. **Top:** Thresholded z-statistic maps of BOLD response analysed for both time points. **Bottom:** Histogram of the BOLD signal change in each MRS voxel (PBR and NBR), for this subject, calculated pre- and post- fMRS acquisition. No considerable change in localisation of PBR or NBR is seen upon visual inspection and the proportion of either response in each voxel appears similar between time-points.

Adequate shimming was achieved in all cases resulting in narrow linewidths. The residual water peak was also lower than total NAA owing to efficient VAPOR water suppression. Also shown in Fig. 5.7 is the LCModel fit of individual metabolites, including the macromolecular resonances (mm) and spectral baselines. Below the metabolite fits, the residual signal is plotted revealing uniform spectral fitting of the metabolites across the spectral window.

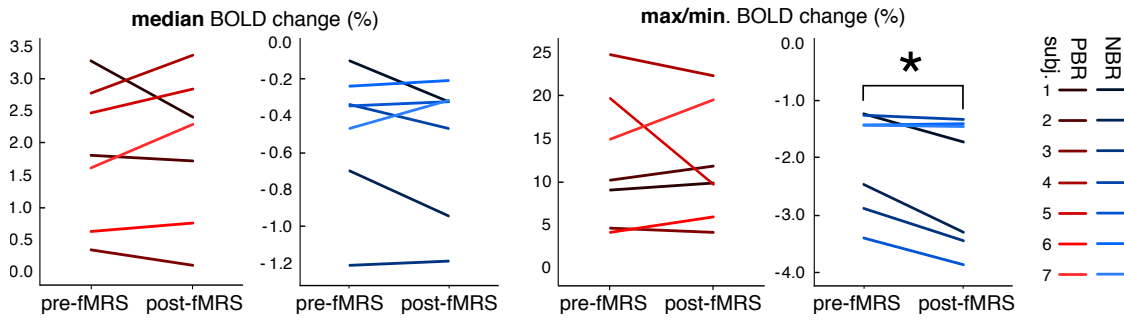


Figure 5.6: Comparison of BOLD response amplitudes across 7 subjects in the PBR and NBR voxels. Changes in the median and maximum/minimum change for PBR/NBR voxels are plotted for first localiser and second localiser carried out after fMRS. No change is evident in the median BOLD response in either voxel. There is a significant difference in the size of the maximum negative response in the NBR voxel, which was 17% larger ($p = 0.029$; two-tailed paired t-test).

Macromolecular contamination

Spectral data from many voxels were observed to contain large macromolecular contamination around 1.7 ppm - attributed to the MM4 resonance [328] (15 / 26 voxels = 58% of the spectra). Since this could not be reliably estimated using the basis set in LCModel, this contamination introduced variations in the estimated baseline signal and, hence, fitting of individual metabolites. An example of this effect can be seen in Fig. 5.8 (top). The broad peak in the raw spectrum at 1.75 ppm is not well estimated by LCModel macromolecular basis, resulting in an uneven baseline across the spectral window (0.5–4.2) ppm. Fig. 5.8 also shows the MRS voxel overlay on a sagittal T_1 -weighted image for this subject. Upon close inspection, the position of the outer edge of the MRS voxel is proximal to lipid rich regions of the dura, both posteriorly and surrounding the cerebellum, which may lead to the contamination in the spectrum and observation of this artefact.

Previous MRS studies of the visual cortex have also been susceptible to significant macromolecular artefacts. In particular, Lunghi *et al.* have noted similar contamination from extraneous lipids and as a result restricted the spectral analysis window to (1.8–4.2) ppm by varying the PPMEND parameter in LCModel [329]. In this functional study, the quantification of the metabolite lactate (with large multiplet resonances beyond 1.8 ppm at 1.3 ppm) was desirable. Therefore, a small region of the spectra

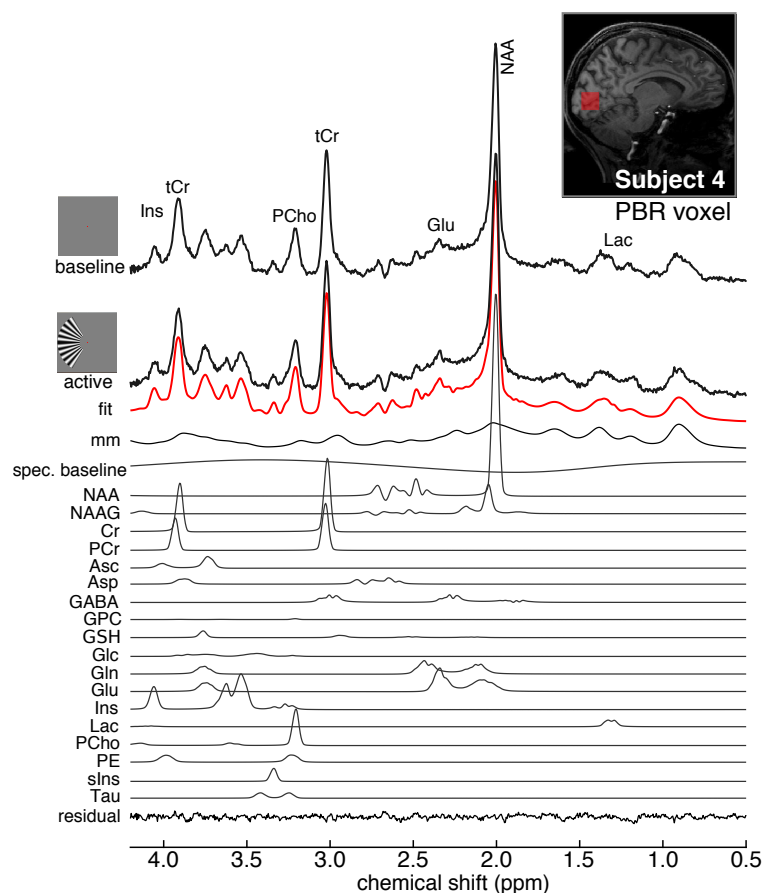


Figure 5.7: Example spectral acquisitions corresponding to baseline and active periods of the visual stimulation (NT = 32). Below the spectra the LCModel fit for the ‘active’ spectrum is plotted. Macromolecular resonances (mm) and the smoothly varying spectral baseline are captured by LCModel. The residual signal (fit-spectrum) reveals comparable fitting across the entire ppm range (0.5–4.2) ppm of interest.

was rejected from analysis only where macromolecular contamination was present. Removal of the contaminated region was achieved using the PPMGAP parameter of LCModel [315]. The LCModel manual suggests that PPMGAP can be used for removing residual water peak (without the need for HLSVD which can introduce artefacts) in the spectra, by inserting a gap in the spectrum. Additionally, PPMGAP is advised for removal of artefacts in GABA-edited spectra from 1.9 to 1.2 ppm, while maintaining peak information beyond this range (0.2 ppm). The spectral range of PPMGAP was manually selected on an individual spectra basis. Fig. 5.8 shows the result of this analysis for a spectrum from a contaminated NBR voxel, with PPMGAP set to range from 1.6–1.9 ppm. It can be seen that the spectral baseline was improved considerably

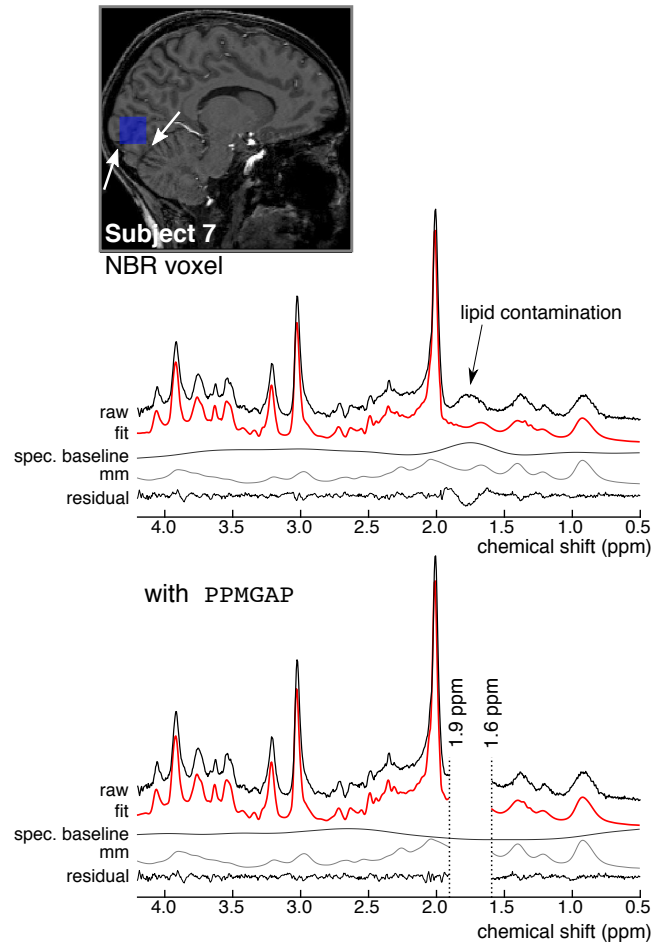


Figure 5.8: Example spectral acquisition showing macromolecular contamination of the spectrum around 1.75 ppm. The outer edge of the voxel is situated close to nearby dura and cerebellum (white arrows). **Top:** LCModel fails to fit the baseline accurately in this subject, which results in poor fitting across the spectral window. **Bottom:** LCModel analysis was, therefore, restricted in the spectral region spanned by the enhanced macromolecular resonance with use of the PPMGAP parameter.

after removal of this resonance from the analysis and resembled that of Fig. 5.7. Table 5.1 gives a summary of all spectra which contained macromolecular artefact and underwent restricted spectral analysis.

In addition to this, one spectrum was rejected (PBR voxel of subject 13) on the basis of poor quality from low signal-to-noise ratio.

Subject	Stimulus	Spectral Contamination			
		PPMGAP			
		PBR		NBR	
		Start	End	Start	End
1	R	-	-	-	-
2	L	-	-	1.5	1.9
3	L	-	-	1.45	1.7
4	L	-	-	-	-
5	L	-	-	1.3	1.7
6	L	1.5	1.8	-	-
7	L	1.45	1.8	1.6	1.9
8	R	1.4	1.7	1.4	1.8
9	R	1.45	1.65	1.45	1.8
10	L	-	-	-	-
11	L	1.6	1.9	1.45	1.65
12	L	1.45	1.8	-	-
13	L	1.4	1.9	1.4	1.7

Table 5.1: Overview of the PBR and NBR voxel spectral quality from each subject. The stimulus lateralisation of left (L) or right (R) is also given for each subject. Where lipid contamination was found to be considerable, the PPMGAP parameter was used in subsequent LCModel analysis. The start and end values (in ppm) are given in the table.

5.4.4 Assessing the effect of BOLD on spectra

The influence of the BOLD effect, due to the increase or decrease of T_2^* on the metabolite linewidths, was examined using the total creatine (tCr) singlet at 3.03 ppm. Since the Lorentzian is the Fourier transform of the monoexponential decay, Lorentzians are often fit to spectral data in the frequency domain, f [330]. A multi-parameter Lorentzian function, L , was thus fit to the tCr peak with variable amplitude, P_1 , centre P_2 , FWHM P_3 and offset P_4 using the LSQCURVEFIT function of Matlab (Mathworks, Inc. Natick MA) of the form

$$L(f) = \frac{2P_1}{\pi} \frac{P_3}{4(f - P_2)^2 + P_3^2} + P_4. \quad (5.2)$$

This automated linewidth analysis method was implemented on combined averaged spectra for baseline and active periods over the entire experiment, without spectral rejection (NT = 64/condition), yielding estimates of the linewidth (P_3) in Hz. Fig. 5.9 shows this fitting for one subject. Fitting was carried out over a range spanning 0.3 ppm (89 Hz, $N_{\text{samples}} = 32$) centred on the creatine peak. Lorentzian lineshapes

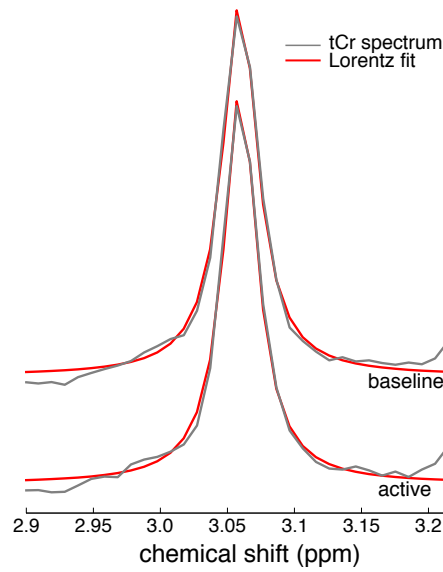


Figure 5.9: Example of Lorentzian line-fitting of the total creatine peak at 3.03 ppm during baseline and active stimulus conditions to estimate degree of line-broadening and narrowing during BOLD response.

were able to fully reproduce the lineshape of the underlying creatine signal and can be seen in Fig. 5.9. Across all spectra, creatine linewidths ranged from 9–15 Hz.

Fig. 5.10 (left) shows the change in the measured creatine linewidths (active–baseline), plotted as a function of the mean BOLD response (% signal change) in each MRS voxel measured during the functional localiser scan. Thus, a decrease in linewidth upon stimulation, due to a reduction in T_2^* , is shown as a negative value in this plot. Subject 1, in which spectra during the final active period were not obtained, was excluded from this analysis to keep the number of acquisitions constant across all subjects. Linewidth differences in NBR and PBR voxels are marked in blue and red, respectively. A linear regression over all linewidth changes reveals a significant relationship between the mean BOLD signal increase and linewidth decreases ($R^2 = 0.49$, $p = 0.003$), as is expected from the increase in T_2^* . The mean creatine linewidth in the PBR voxel showed a significant narrowing upon activation (-0.39 ± 0.45) Hz ($p = 0.006$, one-sided t-test). No significant broadening of the creatine resonance was found in the NBR voxel (0.04 ± 0.35) Hz with $p = 0.3$ (one-sided t-test). There was a significant difference between the NBR and PBR responses (see Fig. 5.10 right) ($p = 0.016$). The analysis was also repeated after excluding one of the subjects (subject 12),

due to apparent anomalous datapoint, (see Fig. 5.10), who experienced a different order of visual stimulation to other subjects. Rejecting this datapoint from the analysis resulted in a significant mean line-narrowing of (-0.31 ± 0.39) Hz ($p = 0.012$) and an average line-broadening in the NBR of (0.12 ± 0.24) Hz, which was close to reaching significance ($p = 0.060$).

The small, marginally significant, trend for broadening occurring in half of the NBR voxel spectra ($n = 6$) indicates the influence of a T_2^* -reduction in negative BOLD during the long stimulus duration of the fMRS paradigm. In addition, subject 5, whose PBR voxel was largely contaminated with negative BOLD response (see Fig. 5.4) during the functional localiser (mean BOLD change = -0.6% , $c = 73\%$), showed a small line-broadening (0.02 Hz) upon stimulation.

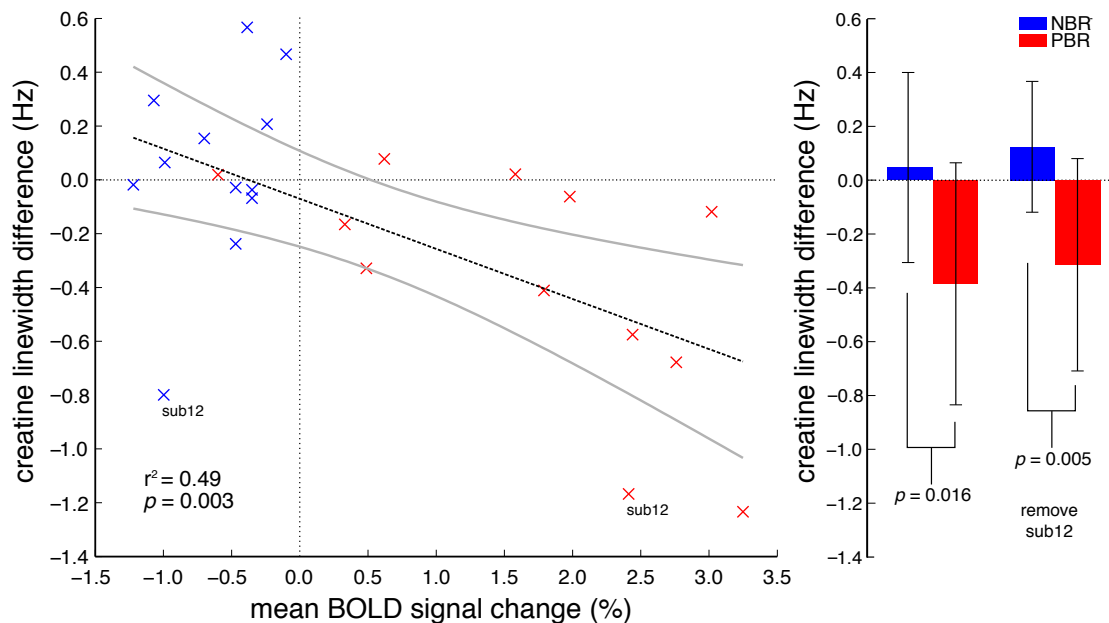


Figure 5.10: Changes in the linewidth of the total creatine peak (3.02 ppm) from baseline to active stimulus conditions, measured within each voxel. **Left:** Correlation of the individual linewidth changes to the mean BOLD signal change in the voxel measured during functional localiser. Data are colour coded corresponding to NBR (blue) and PBR (red) voxels. Data from subject 12, who experienced a different sequence of visual stimulation, are marked (sub12) as an outlier. R^2 of the regression line as well as significance (p) of the gradient is given on the plot. Confidence bands are also given (grey bands) reflecting 95% confidence intervals. **Right:** Mean linewidth changes for NBR and PBR, with and without the inclusion of subject 12, shown with statistical difference.

5.4.5 Neurochemical changes of positive and negative BOLD

In order to assess metabolic changes occurring in the negative BOLD spectra, mean changes in concentration were measured from baseline to active stimulation periods (active–baseline). In total, five PBR spectra and four NBR spectra were excluded from this analysis due to either BOLD contamination or spectral quality, including also subject 1, due to experimenter error. No difference was detected in the mean CSF content in each voxel (PBR : 8.8%, NBR : 9.6%, $p = 0.5$). As previously described, analysis was conducted over the latter half of each block to avoid averaging over transient increases or decreases in neurochemical concentration, thus resulting in baseline and active spectra comprising of 32 spectral averages for each response type. Metabolite concentration changes for the 18 metabolites are summarised in Fig. 5.11 and all changes, including mean CRLBs of fitting (given for baseline spectra), are presented in Table 5.2. All concentrations had a mean CRLB of fitting $< 30\%$, apart from glucose (Glc) which was $< 40\%$.

PBR

In the PBR voxel, lactate showed the greatest concentration increase upon activation relative to the baseline, of $53 \pm 16\%$ ($p = 0.007$) which corresponds to an absolute change of $+0.5 \mu\text{mol/g}$. Additionally, glutamate (Glu) was found to increase significantly during activation ($5 \pm 2\%$, $p = 0.037$) equivalent to $+0.38 \mu\text{mol/g}$. Finally, the concentration of the combined measure of glucose and taurine (Glc+Tau) was found to be reduced ($-16 \pm 5\%$, $p = 0.016$) by $-0.31 \mu\text{mol/g}$. Individual measures of Glc did not show any statistical change ($p = 0.5$), nor were any statistically significant changes in aspartate (Asp) found.

NBR

In the NBR voxel, no statistically significant changes were found for any metabolite, including GABA (5 ± 11 , $p = 0.6$). However, ascorbate (Asc) and inositol (Ins) concentrations both showed trends for a decrease upon stimulation, which approached statistical significance of $-7 \pm 3\%$ ($p = 0.08$) and $-3 \pm 1\%$ ($p = 0.07$), respectively. Furthermore,

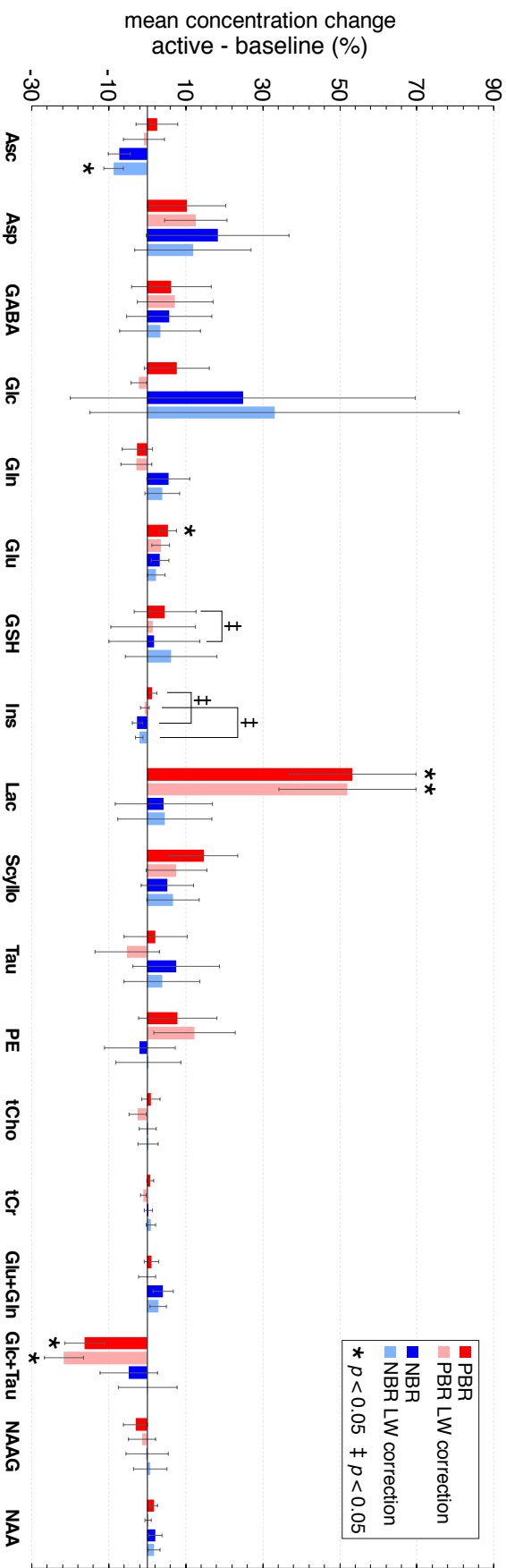


Figure 5.1.1: Mean change (%) in metabolite concentrations across all subjects from baseline to active stimulus condition. Data shown from positive (PBR) and negative BOLD responses (NBR) before and after linewidth (LW) correction. Error bars represent standard error of the mean. * denotes difference between baseline and active conditions with statistical significance $p < 0.05$, (paired t-test). Similarly, † denotes difference between PBR and NBR changes with statistical significance $p < 0.05$ (paired t-test).

changes in Ins in NBR were found to be different from those of the same subject in PBR, where Ins increased on average ($p = 0.007$). Similarly, individual changes in glutathione (GSH) were statistically different between response types ($p = 0.019$) although the mean change was increased upon activation in both PBR and NBR.

	Linewidth Uncorrected										
	PBR					NBR					
Metabolite	baseline conc. ($\mu\text{mol/g}$)	CRLB (%)	mean change (%)	std. error (%)	p-value	baseline conc. ($\mu\text{mol/g}$)	CRLB (%)	mean change (%)	std. error (%)	p-value	PBR vs. NBR
Asc	2.51	11.7	2.51	5.37	0.773	2.96	10.0	-7.27	2.82	0.077	*0.019 *0.007
Asp	3.69	19.9	10.25	10.14	0.412	2.76	30.9	18.26	18.50	0.986	
GABA	4.91	17.2	6.23	10.35	0.974	3.79	19.6	5.74	11.11	0.560	
Glc	1.05	39.3	7.68	8.45	0.541	1.49	31.5	24.84	44.78	0.950	
Gln	7.27	7.6	-2.61	3.92	0.459	6.85	8.2	5.46	5.64	0.411	
Glu	7.06	5.4	5.41	2.24	0.037	6.37	6.3	3.27	2.32	0.243	
GSH	0.82	21.3	4.60	8.07	0.921	0.79	20.2	1.81	11.85	0.674	
Ins	7.08	2.8	1.32	1.23	0.306	7.48	2.9	-2.63	1.28	0.073	
Lac	0.95	22.9	53.35	16.43	0.007	0.94	22.3	4.29	12.59	0.638	
Scyllo	0.22	24.8	14.69	8.84	0.188	0.26	20.1	5.21	6.73	0.504	
Tau	1.15	19.1	2.14	8.18	0.787	1.09	22.3	7.51	11.25	0.975	
PE	2.22	18.1	7.88	10.15	0.691	1.95	24.4	-1.99	9.17	0.874	
PCho+GPC	1.03	4.0	0.93	2.34	0.759	1.14	3.8	0.09	2.16	0.954	
Cr+PCr	8.67	1.3	0.73	0.88	0.447	8.66	1.6	0.25	1.09	0.926	
Glu+Gln	14.33	4.4	1.15	1.86	0.587	13.22	4.8	4.10	2.66	0.218	
Glc+Tau	1.93	17.3	-16.23	5.17	0.016	2.35	15.8	-4.74	7.45	0.495	
NAAG	3.23	12.8	-3.07	3.17	0.395	3.37	16.1	-0.07	5.48	0.876	
NAA	12.85	2.4	1.74	1.00	0.117	12.87	2.7	2.08	1.69	0.221	

Table 5.2: Net concentration changes from baseline upon visual stimulation for PBR and NBR. Mean Cramér-Rao Lower Bounds (CRLBs) are also provided alongside baseline concentration values. The mean change of each metabolite between active and stimulation is averaged across all subjects and significance is assessed using a paired two-tailed t-test. Concentration changes with $p < 0.05$ are shown in bold. No significant changes were detected in the NBR case. The rightmost column indicates those metabolites (GSH and Ins) with significant difference between mean changes of PBR and NBR.

5.4.6 Applying a linewidth correction

To account for the BOLD effect on the spectra [176], a linewidth correction was applied to each spectrum to correct for any concentration differences arising from line-broadening or narrowing measured in Section 5.4.4. Spectra from the active periods of stimulation were broadened or narrowed on a voxel-specific basis using difference values given in Fig. 5.10 by multiplying the FID with a mono-exponential decay function. The identical analysis was then conducted on the metabolite changes with these linewidth corrected spectra.

After linewidth broadening in the PBR, the glutamate increase was no longer statistically significant ($3 \pm 2\%$, $p = 0.21$), whereas functional increases in Lac ($p = 0.023$) and Glc+Tau ($p = 0.002$) concentration remained significant. The NBR voxel showed significant decrease in Asc concentration, after linewidth correction was applied, of $-9 \pm 3\%$ ($p = 0.003$). The Ins decrease was still not sufficient to reach significance $-2 \pm 1\%$ ($p = 0.06$), although a comparison of PBR and NBR changes still revealed a difference between changes of Ins ($p = 0.029$). Changes in GSH were no longer significant between response types. No additional metabolite reached significance after correction for T_2^* -induced linewidth changes.

Linewidth Corrected							PBR vs. NBR
Metabolite	PBR			NBR			
	mean change (%)	std. error (%)	p-value	mean change (%)	std. error (%)	p-value	
Asc	-0.83	5.29	0.681	-8.69	2.56	0.033	*0.029
Asp	12.59	8.15	0.146	11.85	15.16	0.875	
GABA	7.24	9.87	0.930	3.36	10.47	0.710	
Glc	-2.18	2.12	0.488	33.04	47.88	0.779	
Gln	-2.88	4.03	0.394	3.83	4.53	0.512	
Glu	3.49	2.36	0.212	2.29	2.29	0.463	
GSH	1.50	11.04	0.762	6.19	11.96	0.842	
Ins	-0.65	1.19	0.543	-2.04	0.99	0.059	
Lac	52.03	17.79	0.023	4.54	12.20	0.671	
Scyllo	7.54	7.86	0.674	6.71	6.77	0.390	
Tau	-5.24	8.39	0.312	3.84	9.82	0.841	
PE	12.33	10.58	0.403	0.31	8.43	0.888	
PCho+GPC	-2.52	2.17	0.270	0.22	2.59	0.981	
Cr+PCr	-1.00	0.77	0.210	0.91	1.21	0.464	
Glu+Gln	-0.04	2.20	0.932	2.85	2.17	0.327	
Glc+Tau	-21.63	5.02	0.002	0.15	7.59	0.875	
NAAG	-1.43	3.52	0.594	0.79	4.33	0.912	
NAA	0.26	0.83	0.724	1.70	1.55	0.278	

Table 5.3: Net concentration changes from baseline upon visual stimulation for PBR and NBR after linewidth correction from the BOLD effect. Mean Cramér-Rao Lower Bounds (CRLBs) and baseline concentration values are the same as in Table 5.2. The mean change of each metabolite between active and stimulation is averaged across all subjects and significance is assessed using a paired two-tailed t-test. Concentration changes with $p < 0.05$ are shown in bold. Only Asc appears to significantly change by decreasing from baseline in NBR. The rightmost column indicates those metabolites (Ins) with significant difference between mean changes of PBR and NBR.

5.4.7 Metabolite timecourses

The transient responses of the metabolites were analysed over the whole stimulus paradigm using a sliding window analysis, described previously. Subject 12 was

excluded from this analysis after receiving a different paradigm and no data were available for the second active period of subject 1. To compare NBR and PBR metabolite changes, pairwise correlation coefficients, r , between averaged timecourses of NBR and PBR, were also calculated.

Fig. 5.12 shows the sliding window analysis for glutamate (Glu). Individual timecourses are averaged over all subjects generating a mean Glu timecourse. For the PBR response, this mean Glu concentration was observed to reliably increase during periods of active stimulation and decrease during baseline with an amplitude of around $0.3\mu\text{mol/g}$. A similar pattern of response was seen in the NBR, with Glu concentration rising slightly compared to baseline, although the pairwise correlation with the PBR was small and non-significant. Equivalent timecourses were also generated for Lac, Ins, Glc+Tau, Glc, Gln, Asp, GABA, Asc and Cr (see Fig. 5.13).

PBR

The lactate timecourse can be seen to increase slightly, particularly in the final active block, relative to the previous baseline blocks within the PBR. The combined measure of Glc+Tau shows a strong response to the paradigm, with the amplitude of functional decrease around $0.3\mu\text{mol/g}$ matching that expected from the previous analysis of concentration changes. Similarly, Glc concentration also appears to decrease during activation, particularly during the first stimulus block. There appears to be a slight increase in GABA concentration with a sharp decrease to baseline for the first stimulus block.

NBR

Conversely for the NBR, a consistent timecourse for lactate change was not found. Lac appears to increase during the first active stimulation period yet conversely a decrease is observed in the final period. This is in line with the concentration analysis failure to find any concentration difference between the two conditions. Inositol measurement remained constant until the final active stimulus period where they dropped by around $0.5\mu\text{mol/g}$, which may explain the observed trend for decrease found in the previous

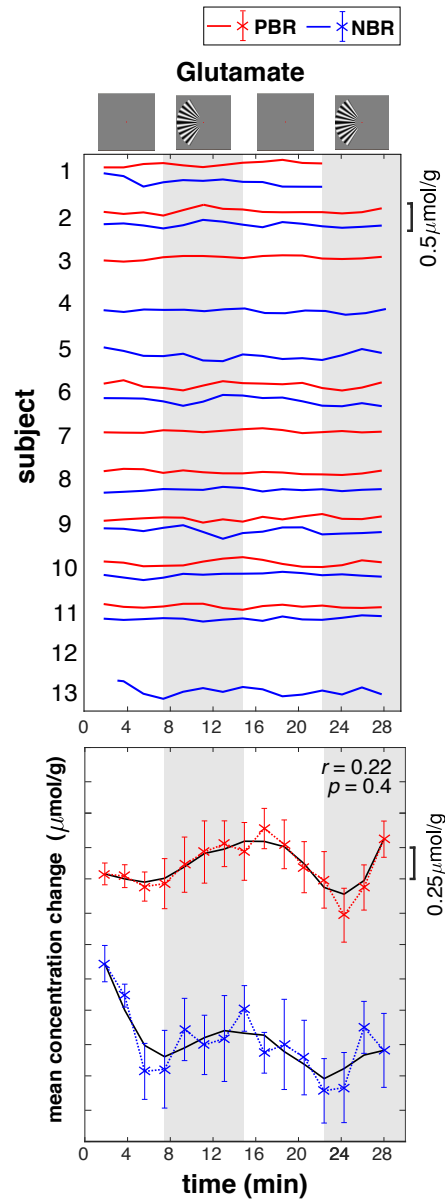


Figure 5.12: Glutamate (Glu) concentration measured over the 30 min fMRS acquisition and averaged across all non-rejected subjects. **Top:** Individual glutamate timecourses measured using sliding window analysis ($N = 16$ transients, window = 8) with concentrations CRLB < 70%. (Subject 1 scan was stopped during last 'active' period). Visual stimulation occurred during 'active' periods highlighted with shaded regions. **Bottom:** Averaged timecourse from all subjects, plotted with standard error and smoothed (black) with window (size = 3). Pairwise linear correlation coefficients between PBR and NBR timecourses, r , and corresponding p -values are provided.

analysis. Similarly, ascorbate (Asc) concentration decreases much more during the second stimulation block, but also decreases in the latter half of the first active period. Aspartate initially appears negatively correlated to the PBR timecourse (decreasing

during the initial active period), however this changed during the second stimulation period. No clear timecourse is observable in Glc or combined Glc+Tau measures. Interestingly, glutamine (Gln) appears to reliably increase from baseline concentrations during stimulation. Furthermore, this pattern of concentration change is negatively correlated to the Gln response in PBR ($r = -0.57$, $p = 0.025$).

5.4.8 Difference spectra

Finally, difference spectra were generated in order to visualise the changes in metabolite concentrations in the spectra. Fig. 5.14 shows the combined spectra for PBR and NBR in active and baseline conditions and shown below each spectrum is the difference signal (active–baseline).

Peaks of tCr and NAA are visible in the difference spectra of both response type above the noise level, reflecting the T_2^* -induced line-narrowing of spectra during the active periods of stimulation. Small residual peaks were also observed in the NBR spectra which also indicate a small T_2^* increase on average. Line-broadening of the active spectra was then applied to correct for the BOLD effect through minimisation of the NAA peak in the residual (0.38 Hz for PBR, and 0.18 Hz for NBR). A Gaussian filter was also applied to remove high frequency noise ($\sigma = 0.1$ s). The residuals do not reveal clear residual metabolite apart from a broad resonance in the PBR which is slightly above the noise level. LCModel was used to fit the residual spectra in both cases with metabolite bases of Glc, Lac and Glu. A residual Lac signal at 1.3 ppm was fit to the PBR residual resulting in a CLRB of 14%. No clear residual metabolite changes above the noise level were observable in the residual of the NBR. Glucose and glutamine resonances failed to fit in either response type.

5.5 Discussion

Negative BOLD responses may reflect neuronal deactivation or regions of active inhibition in the brain. However, neurochemical changes occurring during such responses have not previously been studied in the human brain *in vivo*. If these regions reflect inhibition, the initial hypothesis of this study was that increases in GABA

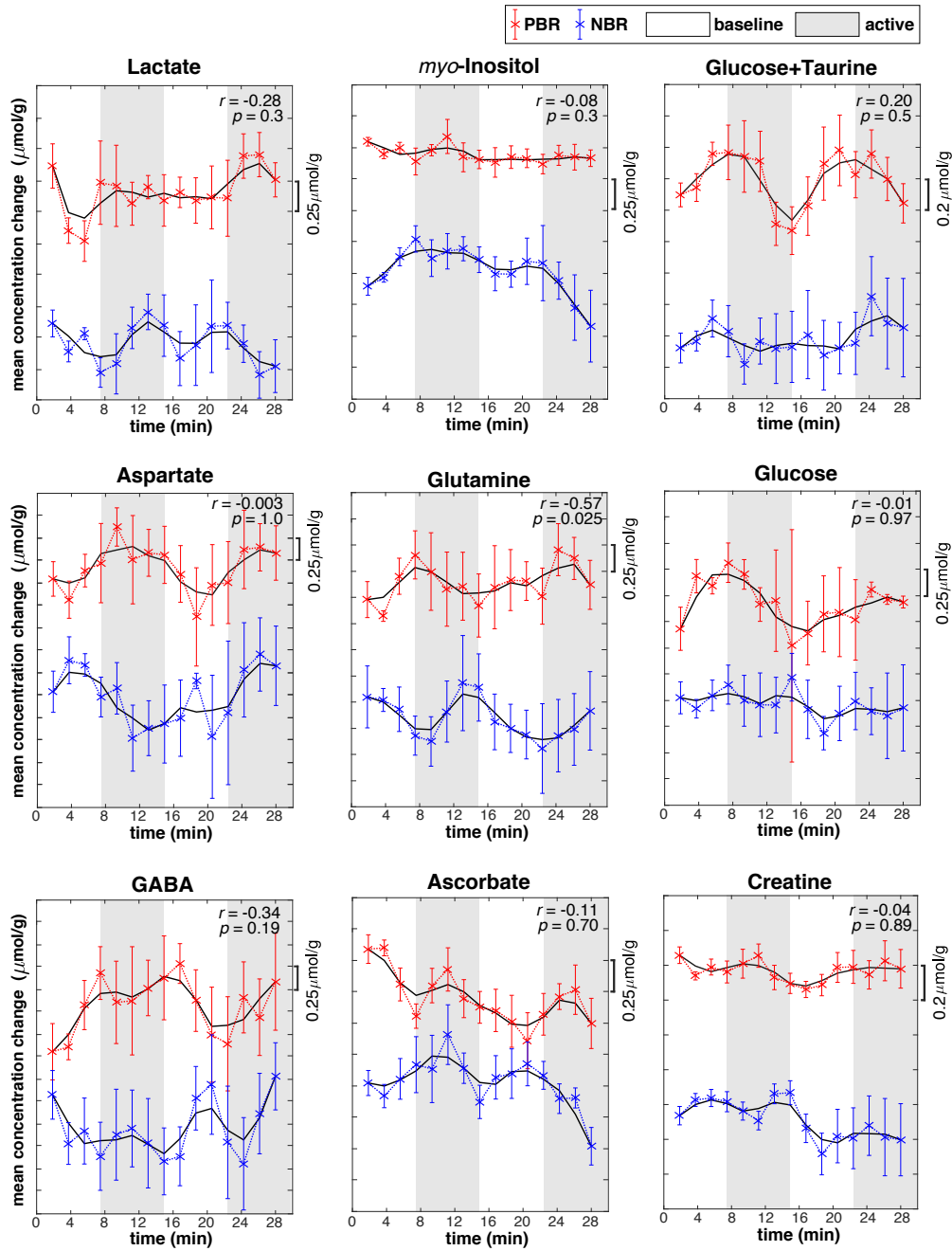


Figure 5.13: Average metabolite concentration timecourses across the spectroscopic acquisition using the sliding window analysis. Visual stimulation occurred during ‘active’ periods highlighted with shaded regions. Timecourses using smoothing window (size = 3) shown overlaid in black. Error bars represent standard errors. Corresponding scale of concentration change shown to the right of each plot. Pairwise linear correlation coefficients between PBR and NBR timecourses, r , and corresponding p -values are also provided.

concentration upon stimulation may be expected, without the increased metabolic demand seen in the positive BOLD response. In order to elucidate the potential

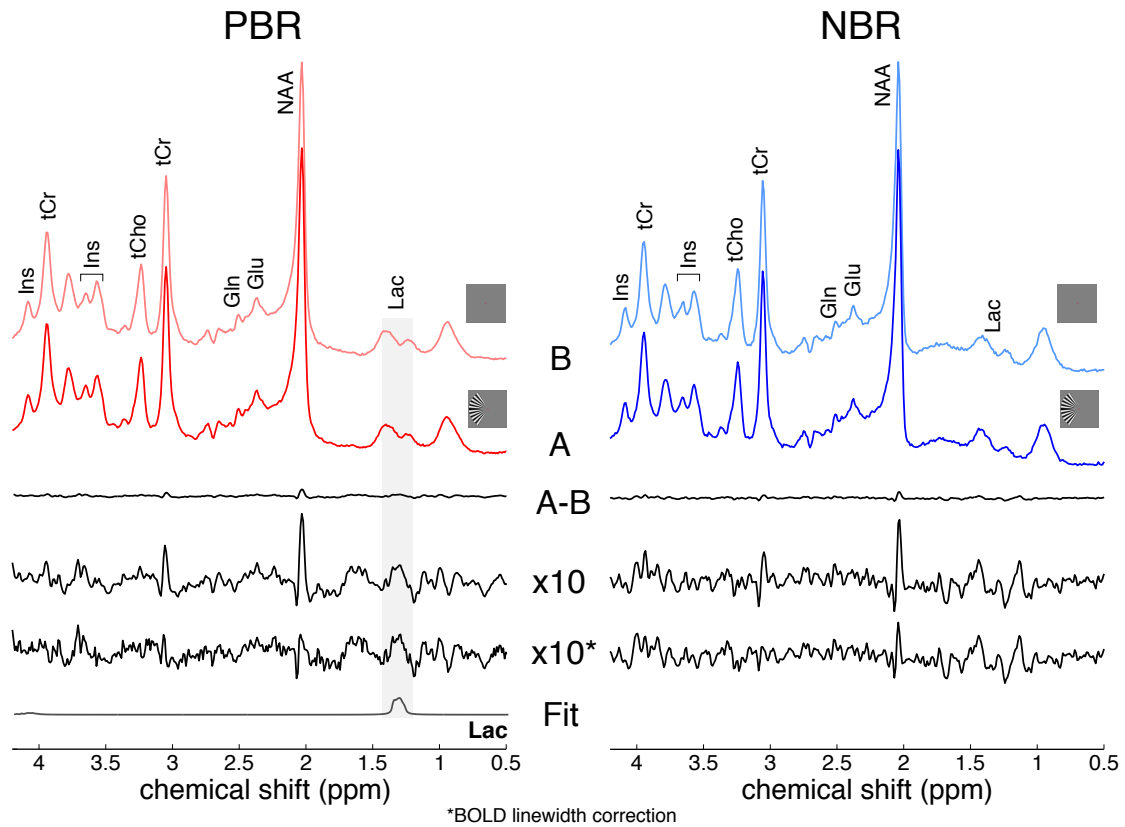


Figure 5.14: Difference between averaged spectra arising from baseline (B) and active (A) periods of the stimulus for PBR (red) and NBR (blue). Residual signal (black line) is the active - baseline (A-B), also plotted below scaled by a factor of 10. Residual peaks of NAA and Cr are visible in the difference spectra in both cases above the noise level. Active spectra are corrected for the BOLD-effect line-broadened by 0.38 Hz in PBR and 0.18 Hz in NBR. No residual metabolite peaks remained above noise level in NBR after correction. A residual Lac signal is, however, visible in the PBR difference spectrum, which is fit with LCModel.

mechanisms and metabolic consequences of this response, and contrast it to the positive BOLD response, neurometabolic changes were probed in the visual cortex using 7 T ^1H -MRS. A novel two-voxel approach, using semi-LASER localisation (Ch. 4), was implemented, able to measure simultaneous changes in positive and negative BOLD regions. A T_2^* -induced line-broadening was observed in some of the spectra arising from negative BOLD regions, however no significant changes in GABA, Lac, Glc or Glu were found during negative BOLD responses using an attentional suppression visual task. Instead a decrease in Asc (9%) was observed, only after correcting for these T_2^* -induced linewidth changes. In contrast, significant increases in Glu and Lac levels were found in the positive response, in agreement with previous findings in

positive BOLD, and reflecting the increased metabolic demand of excitatory synaptic activity in the brain.

5.5.1 Methodology

Visual stimulation in this study was carried out using a modified version of the combined visual attention suppression stimulus previously demonstrated by Gouws *et al.* [1]. BOLD response locations were replicated in areas presented in that study including activation of contralateral V1 and V5. The observation of a subject (subject 5, 5.3C), who demonstrated a large negative BOLD response across both hemispheres, might be attributed to a restricted field-of-view in the scanner. Unexpected regions of contralateral negative BOLD in this subject are retinotopically mapped to the bottom quadrants of the visual field [185]. Thus, if the field-of-view was not symmetric about the midpoint of the stimulus, such a BOLD distribution could be expected.

A limitation of this study is the discrepancy between paradigms run during the fMRI (18 s active) and fMRS (7.5 min active) experiments. Over such long stimulation, as in fMRS, there is the possibility that BOLD signal changes might differ. Changes in the BOLD response present a difficulty, since BOLD amplitude changes correlated with the indirect measure of T_2^* through Cr linewidth changes and the response maps from the functional localiser were used to reject spectra. Previous fMRS studies have, however, also used prolonged stimulation blocks in order to increase spectral averaging and hence SNR for metabolite quantification, with paradigm lengths in the range of ~10 mins [174, 236] and ~5 mins [180]. A possible alternative to this methodology would be to use an interleaved EPI-MRS acquisition strategy, which would allow for simultaneous measurement of the fMRI response together with metabolic changes, similar to that recently proposed by Ip *et al.* [331].

fMRI adaption manifests as a reduction in BOLD signal amplitude after periods of repeated, lengthy stimulation [326, 332]. In this study, a comparison of the BOLD response pre- and post-fMRS did not reveal any significant changes in PBR, as measured by the mean, median and extrema of BOLD changes in the MRS voxel. This is a strong indication that fMRI-adaptation did not occur during the entirety of the

fMRS block. This may be attributed to the randomly changing, unpredictable nature of the peripheral stimulus grating. There was, however, an observable increase in the maximum negative BOLD amplitude relative to the initial stimulus of 17% ($p = 0.029$) with no change in the mean or median BOLD amplitude.

Histograms of the BOLD amplitude in the MRS voxel were slightly skewed towards the negative response type compared to pre-fMRS (Fig. 5.5). An increase in negative BOLD might be a result of an increase in blink events during rest periods towards the end of the experiment. Hupé *et al.* have shown that spontaneous blinking during task can lead to modulation of the fMRI response attributed to the brief changes in luminance [333]. Thus, it is possible that subjects who tire after long periods of lying in the scanner, may have a higher blink rate nearer the end of the scan. Unlike Gouws *et al.*, who measured blink activity and accounted for an increase in blink activity during rest periods on the BOLD data [1], blinking was not measured in the current study. Further work should combine fMRS acquisition with eye-tracking software to account for increases in measured blink activity. Eye-tracking would also ensure that subjects remain centrally fixated throughout the duration of the experiment and as such the location of the BOLD responses is fixed throughout the acquisition.

Due to difficulty in placing the voxels and the nature of the variability in the BOLD responses, this study observed that the majority of MRS voxels contained mixed BOLD responses. In an effort to account for this, a contamination metric and threshold was devised in order to reject spectra that arose from voxels of mixed BOLD response. In the current study, all PBR voxels contained a degree of contamination from the negative response and on average around a quarter (27%) of the significantly responding fMRI voxels were negatively responding. It was not feasible for subsequent analysis to reject data based on the presence of contamination. Instead, outliers greater than one standard deviation were removed, leading to the exclusion of four subjects from the analysis of both response type. Previous fMRS studies have not reported the presence of negative BOLD, although the visual stimuli used in such experiments could potentially generate NBR. This study, therefore, highlights the importance of analysing the BOLD content, or proportion of significantly responding fMRI voxels, within each MRS voxel.

Analysis of neurochemical changes was also hampered by the presence of macromolecular contamination (MM4 at 1.7 ppm) in 58% of the acquired spectra (see Table 5.8). These lipids entered the spectra from neighbouring dura in the cerebellum or outer brain. In the previous chapter (Ch. 4), the identical Hadamard encoded two-voxel method was tested in the occipital lobe of four volunteers and resulted in excellent spectral quality free from contamination. It is suggested that the reason for this discrepancy in spectral quality is that voxel positioning was a manual optimisation between obtaining artefact-free spectra (avoiding lipid regions) and maximising the overlap with the BOLD responses in either voxel. Thus, proximity to dura was often sacrificed for improved BOLD coverage. Macromolecular artefacts around this chemical shift have previously been reported in MR spectroscopy of the visual system [329]. Rather than relying on post-processing techniques, future work could focus on establishing the resonance pattern of macromolecules. This measurement can be achieved, for example, by ascertaining the macromolecular content directly in each voxel placement using an inversion recovery-type sequence previously demonstrated at 7 T [334]. Additionally, switching acquisition techniques to a two-voxel dynamically shimmed MRS scheme may allow for more flexibility in positioning the large MRS voxels in the restricted space of V1, similar to that previously demonstrated in the motor cortex [310]. In the current study placement of the two voxels is restricted along a single coronal plane due to the nature of the multi-banded approach of Hadamard encoding.

5.5.2 Metabolite Changes

This study has indirectly observed negative BOLD in MRS acquisition on the spectral linewidths of several subjects, which arose from the decreased T_2^* content of the voxels. Although the mean linewidth increase in NBR (0.12 ± 0.24) Hz did not quite reach significance ($p = 0.060$) this broadening was observed in 50% of the acquired NBR spectra. Linewidth measurements across both BOLD response types displayed a linear correlation to the strength of the BOLD response, such that line broadening became larger with a more negative response and lines tended to be narrowed more strongly in large positive BOLD responses. This relationship was significant ($p =$

0.003) yet diffuse ($R^2 = 0.49$). Several fMRS studies have previously used the water peak to measure the effect of positive BOLD, and hence a T_2^* increase, on the spectra. Although this was not performed in the current study, linewidth narrowing in PBR has been reported to change to a similar degree in water and creatine [176, 180]. Further studies using the water peak information alone, would allow for more accurate fits of the line broadening, but would also allow measurement of T_2^* values for correction of absolute quantification.

The reasons for the large observed variance in measured linewidth differences (Fig. 5.10) may be numerous. Firstly, BOLD response amplitudes during the localiser and the fMRS scan may not be equivalent since BOLD amplitudes were not measured simultaneously with metabolite measurements. BOLD amplitudes in the linewidth correlation analysis must, therefore, be taken as indirect measures of BOLD content within each MRS voxel. Secondly, linewidths may change over the course of the scan duration. Subject motion may also lead to larger deviations than those expected due to T_2^* effects alone. Thirdly, the negative BOLD response is much weaker compared to the positive response (Fig 5.3). The relative strength of the NBR response is apparent in the difference spectrum, whereby averaging over all NBR voxel spectra resulted in a line-narrowing in NBR similar to the PBR (Fig. 5.14). This observation may be attributed to the fact that NBR spectra from all subjects were included in this analysis, including those previously rejected for BOLD contamination. Thus, the average T_2^* change over all subjects may have been dominated by the stronger PBR response.

With the Hadamard encoded two-voxel MRS technique, metabolic changes in PBR regions were found to be similar to previous studies. In particular, an increase in lactate ($53 \pm 16\%$) and glutamate ($5 \pm 2\%$), above baseline levels was detected before linewidth corrections were applied, which is similar to previous fMRS studies [130, 180, 236]. Lactate increases were similar in absolute terms to those previously reported [169–171]. Timecourses of Lac, Glc and Glu in PBR also matched the stimulation paradigm supporting these findings. The increase in lactate upon activation with concomitant increases in glutamate is attributed to the increased metabolic rate during functional activity. In particular, the increase in Lac and Glu is suggested to support

the interpretation previously suggested of increased pyruvate metabolism through the MAS shuttle (see Section 3.1.4) [179, 180].

However, this study was unable to reproduce the finding of an Asp reduction in PBR regions. Performing the linewidth correction on the data, however, resulted in the loss of significance of this Glu change. Once again, the smaller voxel size of this method, compared to previous literature, may have resulted in reduced sensitivity to such changes. Additionally, in this study, the combined concentration of Glc+Tau, an indirect measure of Glc, was found to significantly decrease ($-21 \pm 5\%$), again in agreement with previous findings [335]. The absence of a significant decrease in Glc concentration alone is indicative of the difficulty in measuring Glc at this echo time. Schaller *et al.* found similar decreases (-12%) in Glc, however they made use of a short-TE (6 ms) localisation scheme where Glc fitting is improved [177].

In their previous 7 T fMRS study, Lin *et al.* noted increases of Glu and decreases of Gln in PBR [236]. Although there was no significant drop in Gln observed in this study, the Gln timecourse in PBR displays a reduction of Gln during periods of activation. Interestingly, the Gln timecourse in PBR was significantly, but weakly, negatively correlated to the timecourse of the NBR response in this study, which showed increases during active periods of stimulation. Observation of an increase in Glu and concomitant decrease in Gln were suggested by Lin *et al.* to reflect an increased flux through the glutamate-glutamine cycle upon activation [236]. An increase in Glu (see Fig. 5.7) with concomitant *increase* in Gln was seen in the NBR timecourse of this study. However, caution must be applied when interpreting the current data as changes in levels of neurotransmission, since Glu also has several metabolic functions. Therefore, it is hard to draw firm conclusions without a rigorous analysis of the reaction kinetics of synthesis and catabolism.

5.5.3 Not GABA rather Asc?

In this study, there was no evidence for changes in the concentration of inhibitory neurotransmitter GABA within NBR. This, therefore, suggests that negative BOLD in the visual cortex may not occur with a concomitant increase in GABA above baseline.

Still, it is not possible to entirely rule out the initial hypothesis that GABA may play a role in negative BOLD due to a number of reasons. Firstly, the question remains as to whether measures of GABA concentration with ^1H -MRS would be able to measure small 'phasic' or transient GABA changes over the timescales in this work [228]. Rapid changes in GABA have been reported using MRS in previous human studies over periods of 30 mins [336]. In addition the rate of GABA synthesis has been estimated at around $0.14\mu\text{mol/g/min}$ using ^{13}C labelling in rat [337], thus any short term changes in GABA concentration over the timescales presented here may prove difficult to measure. In this study, the variation of GABA change across all subjects reached $\pm 0.5\mu\text{mol/g}$ (standard error). Sensitivity to any GABA change may therefore be limited, and further worsened by the fact that NBR amplitude is much weaker compared to positive BOLD changes.

In addition, compared to Bednarik *et al.* the voxels used in this study were half the size in volume, which resulted in a penalty of 50% less signal amplitude to detect such changes [180]. Finally, it could be true that tonic, rather than phasic changes in GABA, play a stronger modulatory role in BOLD responses through selective release of existing GABA from the total available pool [229]. Unlike glutamate, the response to GABA uptake in astrocytes may not generate a metabolic signature which contributes to the BOLD response [219]. The absence of changes in metabolites Lac and Glu may also support this.

The only statistically significant change in NBR occurred after correcting for BOLD-linewidth change was that of Asc (reduction of $(-9\% \pm 3\%)$). The Asc timecourse in the sliding window analysis (Fig. 5.13), also reveals a sharp decrease during the latter half of each stimulation block, particularly for the final block. Aside from its protective function (vitamin C), ascorbate has been linked to several neuromodulatory functions and exists in large quantities in the brain [338]. Extracellular ascorbic acid has been previously shown to increase during glutamatergic activity [339]. Glutamate release and uptake has also been linked to the release of ascorbate [340, 341] and Asc may even act as a 'switch' to inhibit glucose utilisation in neurons during glutamatergic functional activity allowing lactate as a substrate [342]. Furthermore, it is interesting

to note that decreases in Asc have also been detected when pharmacological agents are applied that enhance GABA-mediated inhibition [343]. Therefore, it is tentatively suggested that the observed Asc decrease in NBR may be indicative of a reduction in excitatory glutamatergic activity. However, it should be noted that the brain has no means to produce Asc. Although Asc has previously been shown to be detectable at 7 T with short echo (6 ms) STEAM localisation, its detection is made challenging by neighbouring resonances around 3.73 ppm of Glu, Gln, GSH [313, 344]. In addition, significance of Asc change in the present study appeared only after linewidth correction was applied, although Asc was near significance ($p = 0.07$) before correction. Further work is needed to verify that the semi-LASER (36 ms) sequence used here can reliably identify Asc. The use of difference editing techniques or short echo time spectroscopy may be needed to confirm the validity this finding.

5.6 Conclusion

A novel functional MRS study of the negative and positive BOLD response in human brain has been carried out for the first time in thirteen volunteers at 7 T. Using a simultaneous two-voxel MRS acquisition strategy, combined with a visual attentional suppression task, metabolite changes were measured in both response types. Linewidth broadening, due to T_2^* of individual spectra was found to occur in half of the negative BOLD response regions upon activation and was significantly different to the narrowing observed in the positive BOLD response. Lactate and glutamate concentrations were found to significantly increase in positive BOLD responses consistent with previous findings and corresponding to increased rate of oxidation during functional activation. However, no significant changes were found in the negative BOLD response apart from a decrease in ascorbate concentration, which reached significance after applying a BOLD-induced linewidth correction (-9%). Together the findings of this study suggest that the negative BOLD response may simply reflect a decrease in glutamatergic activity, without observation of neurochemical changes associated with the inhibitory neurotransmitter GABA or lower metabolic demand. An increase in statistical power is required to verify the findings of this study.

6

Improved detection of 2-HG at 3T

The development of an optimised semi-LASER sequence for the detection of the tumour metabolite 2-HG at 3T is described in the following chapter. Firstly, the use of MRS for detection of 2-HG is reviewed and the challenges in terms of modified spectral profile and chemical shift displacement discussed. An optimisation of the echo time of a semi-LASER scheme and subsequent analysis of the 2-D localisation performance using density matrix simulations is presented. This optimised sequence is compared throughout to an existing PRESS localisation scheme, which suffers from large chemical shift displacement [345]. Both sequences are then compared *in vivo* in 11 patients with glioma to assess robustness of 2-HG detection. Finally, the effect of GABA overlap on spectral quantification is discussed. The majority of this chapter appears in previously published work of Berrington et al. (2016) [346].

6.1 Introduction

Personalised medicine in the treatment of cancer calls for the discovery of reliable biomarkers and the development of tools capable of differential diagnosis according to genetic subtypes. In brain tumours, accumulation of the ‘oncometabolite’ 2-HG (D-2-hydroxyglutarate), in the majority (~80%) of grade II-III gliomas and secondary glioblastomas [242, 264] is potentially such a biomarker. 2-HG is produced as a metabolic product of somatic mutations in genes encoding for isocitrate dehydrogenase (IDH) [264], specifically IDH1 and IDH2, which encode for enzymes located in the cytosol and mitochondria respectively. Recent studies have shown that glioma patients with the IDH mutation have an improved prognosis compared to those without [244, 288], and that stratification by IDH mutation status and its biochemical consequences may offer new opportunities for the diagnosis, monitoring and treatment of glioma [347, 348]. Accurate, non-invasive and robust determination of 2-HG in tumours, as a

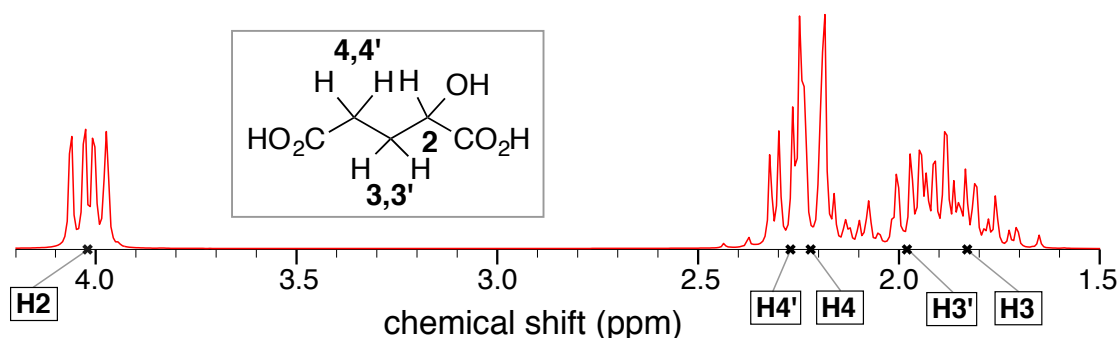


Figure 6.1: Proton spectral pattern of 2-HG (2-hydroxyglutarate) and corresponding hydrogen spins. Shown here at 3T (127.7 MHz) with 1 Hz line broadening, 8912 spectral points and 6 kHz spectral width. Only contributions from non-exchangeable, J-coupled spins at positions 2, 3 and 4 are visible [350]. Spectrum is from 2-D density matrix simulations of 2-HG using a pulse-acquire experiment.

surrogate marker for IDH mutation status, is therefore key for pre-surgical diagnosis and subsequent monitoring of treatment efficacy and relapse.

6.1.1 Previous attempts to detect 2-HG

Three decades ago it was shown that an accumulation of 2-hydroxyglutaric acid¹, in the genetically acquired metabolic disease L-2-hydroxyglutaric aciduria, could be detected in urine using ¹H-NMR spectroscopy [349]. It was not until 2002 that an accurate study of the chemical shifts and J-coupling constants was carried out for the purpose of 2-HG detection; the 2-HG molecule has a strongly coupled AMNPQ spin system with five non-exchangeable proton resonances (H2, H3, H3', H4, H4') at chemical shifts of 4.02, 1.83, 1.98, 2.22 and 2.27 ppm at pH 7 [350] (see Fig. 6.1).

2-HG has since been shown to be detectable in tumours using MR spectroscopy, initially using *ex vivo* HRMAS (high resolution magic angle spinning) NMR [351], thereby opening up the possibility of IDH-mutation identification *in vivo* without the need for invasive surgical biopsy.

The detection of 2-HG using *in vivo* MRS, however, brings with it several technical challenges. Initial work by Pope *et al.*, made use of a short echo time (TE = 30 ms) 1H-MRS PRESS [67, 68] sequence at 3T to demonstrate 2-HG detection *in vivo* [352]. However, spectral overlap between the 2-HG peak at 2.25 ppm (H4 and H4') and

¹2-hydroxyglutarate (2-HG) is simply the conjugate base of 2-hydroxyglutaric acid

glutamate (Glu) and glutamine (Gln) resonances, in addition to the large baseline offset at short echo time, led to a large false positive detection rate for 2-HG. Interestingly, even at 9.4T (where spectral resolution is around 3 times greater), accurate short-TE detection of 2-HG in mouse models remains challenging [353], although recent success has been reported in patient studies [354]. To overcome this spectral overlap issue, *in vivo* 2D correlation spectroscopy (2D-COSY) and spectral editing with MEGA-LASER have been proposed by Andronesi *et al.* [355]. However, such techniques can suffer from longer scan times, which are challenging to implement in clinical studies. J-editing with MEGA-LASER on the H2 and H3–H3' scalar coupled spins results in a difference spectrum with a clear 2-HG peak at 4.02 ppm; however, in addition to the dependence of such sequences on editing efficiency, localised shimming as used in single voxel MRS of tumours can introduce spurious water echoes around this chemical shift value [314], which could negatively influence difference spectra.

An alternative to these approaches is to use single shot techniques with longer echo times. Choi *et al.* have optimised the timings of a PRESS sequence (TE = 97 ms) to maximise the H4/H4' peak of 2-HG at 2.25 ppm amongst the Glu and Gln overlaps and were able to differentiate between IDH mutant and wild-type tumours with much higher accuracy than with short TE PRESS [345, 356].

6.1.2 2-HG spectrum is modulated by chemical shift displacement

The spectra of J-coupled metabolites are susceptible to signal modulation using PRESS localisation, through imperfect slice selection profiles and chemical shift displacement artefact arising from the use of narrow bandwidth refocusing pulses [357, 358]. In a simulation study by Kaiser *et al.*, the effect of poor localisation from chemical shift displacement artefact was shown to dramatically affect the resonance patterns of the coupled metabolites Lac, Glu, NAA and Ins [359]. For 2-HG, this may offer a potential explanation for an observed signal loss of the H3–H3' resonance (1.9 ppm) when using the 97 ms PRESS sequence [356].

Detection of 2-HG at 3 T may, however, benefit from the presence of this peak at 1.9 ppm. Yet, this chemical shift position also lies near Glu and Gln resonances

as well as a γ -aminobutyric acid (GABA) resonance at 1.89 ppm, and is adjacent to the NAA peak at 2.01 ppm [313].

This study, therefore, investigates the benefit of using broadband adiabatic refocusing pulses at 3 T. Such a scheme may reduce signal loss associated with the combination of poor slice localisation and chemical shift displacement for the detection of the coupled spin system of 2-HG, which may ultimately lead to improved detection of this novel biomarker.

6.2 Aim

An optimised long-TE semi-LASER localisation scheme [20] was compared to an existing PRESS sequence (TE = 97 ms) to detect 2-HG at 3T in a phantom and *in vivo*. In particular, optimisation was performed on the 1.9 ppm resonance of 2-HG, which is not present when using PRESS localisation [356]. Quantum mechanical density matrix simulations and phantom validations were carried out using both semi-LASER and PRESS (TE = 97 ms) schemes to assess the benefits of adiabatic localisation on the 2-HG spectral profile. The sensitivity of the optimised method was then compared in a phantom before being tested *in vivo* in eleven glioma patients with and without IDH-mutation.

6.3 Optimisation of semi-LASER TE for 2-HG

The TE of a semi-LASER scheme is firstly optimised for the detection of the H3–H3' multiplet at 1.9 ppm amongst the commonly observed overlapping metabolites of Glu and Gln.

The implementation of semi-LASER in this work, is one previously reported by Scheenen *et al.* [76], with the two pairs of gradient selective refocussing pulses positioned in a symmetrical manner (Fig. 6.2). The total echo time is defined by the duration of the 90° and 180° refocusing pulses, t_{90} and, t_{180} and inter-pulse

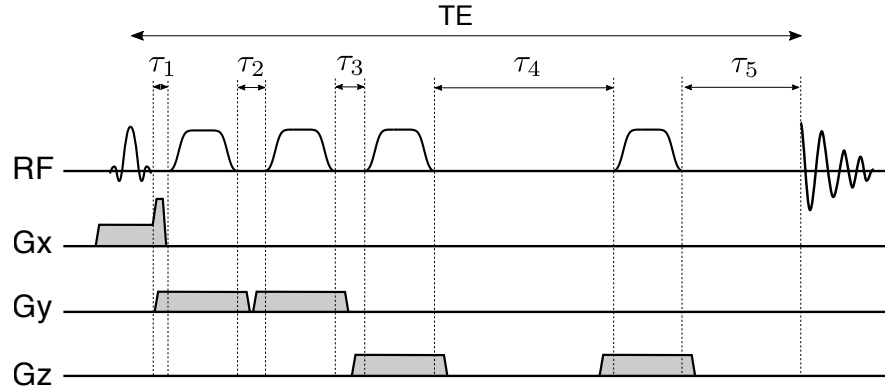


Figure 6.2: Pulse sequence diagram for semi-LASER implementation. The total echo time (TE) is defined using inter-pulse delays τ ; timings are given in the text. Crusher gradients are included in the sequence, but not shown above.

durations τ , such that

$$\tau_1 = 0.78,$$

$$\tau_2 = 1.56,$$

$$\tau_3 = 1.56,$$

$$\tau_4 = (TE - 4t_{180} - 2\tau_3)/2,$$

$$\tau_5 = TE - \tau_1 - \tau_2 - \tau_3 - \tau_4 - 4t_{180} - t_{90}/2,$$

in milliseconds. Modification of TE, is therefore equivalent to varying the onset time of the final refocusing pulse (τ_4) and readout (τ_5).

The effect of TE variation on the spectral profile of 2-HG, Glu and Gln is evaluated by density matrix simulations. Echo times are varied in 5 ms increments ranging from 30 ms to 135 ms. In order to capture imperfections associated with RF localisation, real refocusing pulses are included in the simulations. Excitation pulses are also modelled as a hard 90° nutation. In the semi-LASER sequence, refocusing is carried out using HS4 R25 adiabatic pulses (duration = 4.5 ms, bandwidth = 5.5 kHz), chosen for their large bandwidth and insensitivity to B_1 inhomogeneity [60].

In addition to simulating the effect of echo time on 2-HG, overlapping spin-systems of glutamate and glutamine are also included with chemical shifts and coupling constants based on previously reported values [313]. The GAMMA/PyGAMMA simulation

library of VeSPA (Versatile Simulation, Pulses and Analysis) is chosen to perform the simulations [309].

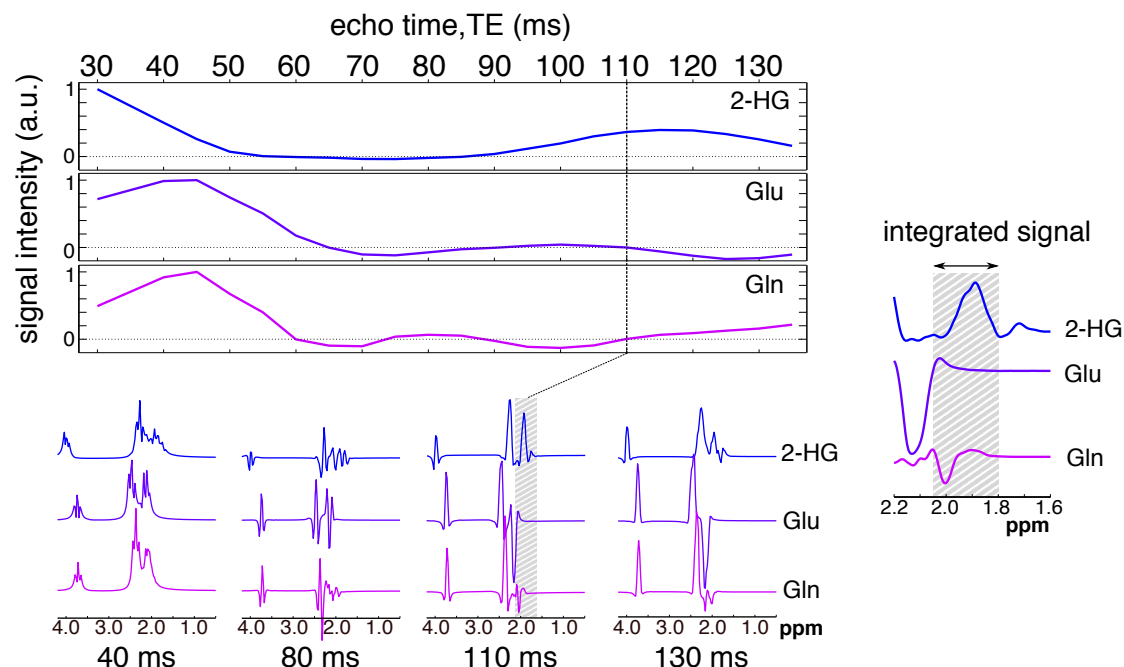


Figure 6.3: Optimisation of semi-LASER echo time for the detection of 2-HG. **Top:** Signal integral is plotted as a function of echo time (TE) for 2-HG, Glu and Gln. **Bottom:** Spectral profiles shown for four different echo times. **Right:** Close up of 2-HG H3–H3' (1.9 ppm) resonance at 110 ms with overlaps showing 15 Hz integration window. At 110 ms, semi-LASER generates near zero overlap from Glu and Gln around 1.9 ppm.

The integrated signal intensity over a 15 Hz window centred on 1.91 ppm is shown for these three metabolites at increasing echo times (Fig. 6.3 A). It can be seen that at TE = 110 ms, the H3–H3' (1.9 ppm) peak has an upright lineshape, while overlapping signal contributions from both Glu and Gln are almost zero (Fig 6.3 B and C). Signal yield of 2-HG is higher at short echo times (42% at 40 ms), though multiplet lineshapes and the similarity of the spectral patterns of Glu and Gln do not allow clear distinction from 2-HG. The echo time of semi-LASER was, therefore, selected as 110 ms to elucidate 2-HG from these overlaps.

6.4 Simulations of 2-D localisation

In order to analyse the effect of chemical shift displacement and slice selection on the spectral profile of 2-HG, custom 2-dimensional quantum mechanical density matrix

simulations were developed and written in Matlab (Mathworks, Inc., Natick, MA).

Several methods exist to simulate localised spectra; as the accuracy of a particular simulation method improves there is generally a penalty of increased computational demand. Aside from hard-pulse approximations, the simplest method is a four-compartment analysis [360], which accounts solely for the effect of frequency-based localisation, i.e. chemical shift displacement artefact, on J-coupled spin systems. Flip angle profile models [361] additionally characterise the effect of the imperfect transition bands of frequency selective RF refocusing pulses. Lastly, complete evolution of the spin-system during the sequence, including that which occurs during the RF pulse itself, can be simulated [46, 357].

In comparative work carried out by Choi *et al.* [356], the incorporation of real RF pulse and gradient information in density matrix simulations accurately captured the observed spectral profile of 2-HG in a phantom and, in particular, the loss of resonance at 1.9 ppm. In the following work, localisation of the 2-HG spin-system was initially simulated using a flip-angle profile approach in order to capture this behaviour, with the hope of reducing the required computational complexity of simulation.

6.4.1 Failure of the flip-angle profile approach

In the flip-angle profile approach, refocussing pulses are modelled as instantaneous pulses. The evolution due to chemical shift and J-coupling occurs only during the inter-pulse durations. To begin, the simulated magnetisation profile over each refocussing dimension, following a slice-selective RF pulse, is modelled using the Shinnar-Le Roux algorithm [320]. This algorithm is chosen because of its simplicity and the ease of incorporating gradient information. The resulting magnetisation vector as a function of spatial position, $\vec{M}(r) = (M_{xy}, M_{xy}^*, M_z)$, is then used to calculate the effective flip angle, θ , where $\theta(r) = \arctan(M_z(r) / |M_{xy}(r)|)$. The centre of this flip-angle distribution is shifted as a function of the chemical shift of each spin in the system. Product operators for chemical shift evolution and J-coupling are calculated, and then at each spatial position across a 2-dimensional grid, the Hamiltonian associated with RF pulse is generated based on the flip angle experienced by each spin at each spatial

position. This method, therefore, characterises the sidelobes and transition bands of the refocussing profile as well as any compartmental artefacts associated with the chemical shift displacement of J-coupled spins.

After simulating with this method, the flip-angle model failed to fully capture the complete spectral profile of 2-HG when compared to phantom data. Appendix E shows an example of this along with a visual comparison of the simulation methods. As Maudsley *et al.* have duly noted, and was later realised in this work, the effect of chemical shift and J-evolution during a pulse is non-negligible when $1/J$ is comparable to the pulse duration [46]. The strongest J-coupling in 2-HG, between the H4 and H4' spins (15.0 Hz), is $1/J_{44'} = 67$ ms. For two PRESS Refoman6 refocusing pulses (duration = 13.2 ms), this corresponds to nearly 40% of the J-evolution time. Thus, the localised simulations of 2-HG must also account for coupling and chemical shift evolution during the refocusing pulses.

6.4.2 Complete evolution approach

To simulate complete evolution of the 2-HG spin-system, similar to the the flip-angle profile approach, density matrix product operators were calculated at each stage of the sequence corresponding to Hamiltonians for J-coupling, RF pulse and chemical shift evolution. In the following approach, however, the refocusing pulses are divided into a series of small hard pulse operators, interspersed with J-evolution and spatially dependent chemical shift operators, to simulate slice selection gradients. The 90° excitation remained hard with zero duration.

Crusher gradients were simulated as incremental $\pi/4$ rotations about the z-axis positioned around each refocusing pulse similar to previous work [359, 362]. To match *in vivo* acquisition, the RF carrier frequency was set to 2.7 ppm and all simulations were line broadened to 6 Hz by multiplication of the resulting FID with an decaying exponential factor. The sampling frequency was set at 6 kHz and a total of 8192 time points were sampled.

These complete 2-D localised simulations were carried out using the optimised semi-LASER sequence and the previously demonstrated asymmetric PRESS sequence

Parameter	PRESS	semi-LASER
Echo Time (TE) / <i>ms</i>	97 (32, 65)	110
Refocusing pulse	Refoman6	HS4 R25
Duration / <i>ms</i>	13.28	4.5
Bandwidth / <i>kHz</i>	1.24	5.5
Max B ₁ Amplitude / μT	13.4	28
Number of Points	200	511
Spectral Width / <i>kHz</i>	6	
Voxel Size / <i>mm</i>	10	
Number of Samples	8192	
Grid Size	200×200	

Table 6.1: Parameters for the 2-D density matrix simulations of PRESS and semi-LASER localisation, including refocusing pulse information for both sequences.

[345] (TE = 97 ms; TE₁ = 32 ms; TE₂ = 65 ms; sequence diagram in Appendix A). Information on the refocussing pulse characteristics of PRESS and semi-LASER as well as the subsequent simulation parameters are given in Table 6.1. In the ideal simulations, RF refocussing pulses are modelled as hard instantaneous 180° pulses.

2-HG was simulated, along with 20 other commonly observed tumour metabolites. Spin systems were simulated on a 200×200 grid, with a resolution of 1% of the nominal voxel size. For larger spin systems ($N \geq 6$) the grid resolution was reduced to 20×20 resolution or 5% of the nominal voxel size to reduce computational time. Simulations were run on a computing cluster with a minimum 2.67 GHz processor and 64GB RAM.

The following is pseudocode which describes the implementation of the density matrix simulations for 2-D localisation of 2-HG.

6.4.3 Localisation with PRESS and semi-LASER

The localisation of the 2-HG H3–H3' multiplet was firstly investigated using simulations of the optimised semi-LASER sequence (TE = 110 ms) and the original PRESS (TE = 97 ms) sequence [345] (Fig. 6.4A). The total integrated H3–H3' peak 2-HG intensity at 1.9 ppm, following 2-D fully localised simulations (green), is shown at each position on the 200x200 simulated grid on the intensity maps in Fig. 4A.

Pseudocode of localisation using 180_x° pulse and free evolution

```

N = 200                                ▷ Set the number of 2D grid points
σ0 = initial density matrix
pulse = read in RF pulse information
for x = 1 : N do
  for y = 1 : N do                                ▷ Loop over spatial grid

    ωx,s = δs[Hz] + γGx/2π
    ωy,s = δs[Hz] + γGy/2π                    ▷ Frequency offset of spin s at x, y with shift δ

    for c = 0 :  $\frac{\pi}{2}$  :  $\frac{3\pi}{2}$  do                                ▷ Crusher Gradients
      σc ← e-iHcrush(c) σ0 eiHcrush(c)
      for n = 1 : length(pulse) do                                ▷ Loop over points in 180° pulse
        σc ← e-iHRF(n)tRF σc eiHRF(n)tRF                    ▷ RF evolution at point n for tRF
        σc ← e-iHCS(ωx,s)tRF σc eiHCS(ωx,s)tRF                ▷ Chemical shift at x, for tRF
        σc ← e-iHJtRF σc eiHJtRF                                ▷ J-evolution for tRF
      end for
      σc ← e-iHcrush(c) σc eiHcrush(c)
      σ += σc
    end for
    σ ← σ/4                                ▷ Normalise after 4-fold cycle of crusher gradients

    σ ← e-iHfreeτ σ eiHfreeτ                                ▷ Free evolution for τ
  end for
end for

```

A heterogeneous distribution of signal intensity at 1.9 ppm, which manifests as compartments of several distinct signal profiles, is seen using PRESS localisation. In particular, there is complete inversion of the 1.9 ppm peak within one of these compartments (Fig. 6.4A(1)), accounting for ~19% of the total 2-D voxel size, which has acquired an antiphase signal characteristic compared to the ideal pulse simulation (grey). Only around 52% of the localised PRESS voxel contains a positive signal contribution at this shift value (Fig. 6.4A(2)). The semi-LASER sequence, however, displays a far superior slice selection profile and reduced chemical shift displacement artefact for the multiplet of H3–H3'. This is evident from the presence of a single uniform compartment, containing positive signal contribution, and almost identical to the ideal pulse simulations (Fig. 6.4A(3)).

Fig. 6.4B shows the spectral comparison of 2-HG and Gly simulated using ideal

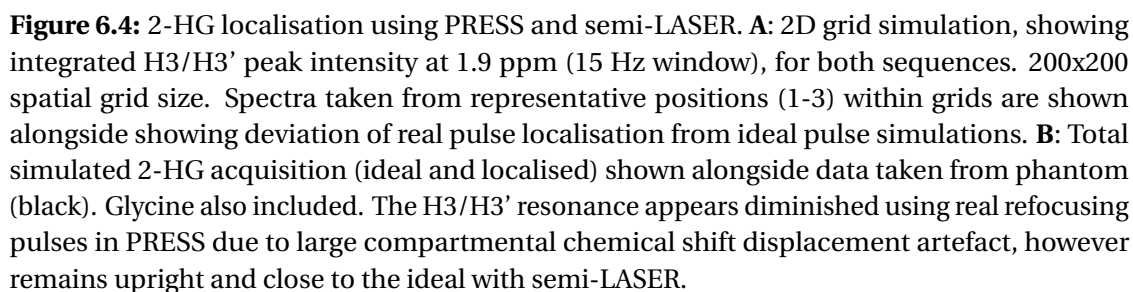
(grey) and fully localised simulations (green) and the spectrum acquired from a phantom test bottle containing 2 mM 2-HG and 10 mM Gly (black) (Section 6.5). Using PRESS localisation there is a loss of the H3–H3' resonance as shown in both the localised simulations and phantom data, which is in strong contrast to ideal simulations. This peak is preserved using semi-LASER at TE of 110 ms, which generates near identical spectral profiles across ideal, localised and phantom data.

Comparing the fully-localised 2-HG spectra to a simulated pulse-acquire experiment (TE = 0), the signal amplitude at 2.25 ppm is measured to be 53.5% using PRESS and 37.9% using semi-LASER (neglecting T_2 -relaxation). At the 1.9 ppm resonance (optimised for in this study) this becomes 2.9% for PRESS and 36.4% for semi-LASER.

6.5 Assessing the sensitivity of detection in phantom

To test the sensitivity of the optimised semi-LASER method, a test phantom containing six 66 ml bottles of varying 2-HG concentrations (or R,S- α -hydroxyglutaric acid, Sigma-Aldrich) was constructed. 10mM of glycine (Gly) was included in each bottle to act as a reference peak, having only a singlet resonance at 3.55 ppm [313]. Concentrations of 2-HG were 0.1 mM, 0.5 mM, 1 mM, 2 mM, 5 mM and 8 mM. Each bottle was buffered with PBS buffer and average pH was measured to be 7.5 ± 0.1 . The larger bottle was filled with NaCl solution. Phantom measurements were acquired on a 3T whole body MR Prisma system (Siemens, Erlangen) with 32-channel receive array head coil. Voxels of $(20 \times 20 \times 20)$ mm³ volume were obtained and shimmed to second order using GRESHIM [311]. Both PRESS (TE = 97 ms) and optimised semi-LASER (TE = 110 ms) spectra were acquired with VAPOR water suppression and outer volume saturation bands [301] at a TR of 3 s. RF pulses and timings were equivalent to those in simulations and 128 transients were acquired for each bottle in the phantom together with an unsuppressed water reference used for eddy current correction. The excitation pulse used in the subsequent acquisitions of semi-LASER and PRESS is a hsinc 90° pulse (duration = 2.56 ms, bandwidth = 3.4 kHz).

The sensitivity of both methods to detect 2-HG across a range of concentrations was next assessed in the phantom. Spectra were line-broadened (6 Hz) to match *in*



To further examine the sensitivity of both methods, data were split into averaged spectra containing 2, 4, 8, 16, 32, 64 and 128 transients. Fig. 6.5A also shows the LCModel CRLB output as a function of the acquired number of averages, using the 2 mM 2-HG phantom bottle as an example. CRLBs from both methods display identical

relationships with increasing number of spectral averages. Even with only 2 spectral averages ($\text{SNR} \sim 15$) both methods fit 2-HG at 2 mM with CRLBs of $(15 \pm 7)\%$ and $(14 \pm 6)\%$ for PRESS and semi-LASER, respectively. The detected concentrations were then compared in Fig. 6.5B. The ratio of detected 2-HG to Gly is plotted over the same concentration values, without correcting for T_2 differences. For concentrations greater than 0.1 mM (CRLBs $< 20\%$) both methods were significantly different in their estimation of 2-HG concentration ($p < 0.008$, paired t-test) with average detection ratios of $(76 \pm 6)\%$ and $(96 \pm 6)\%$ of the true ratio for PRESS and semi-LASER, respectively.

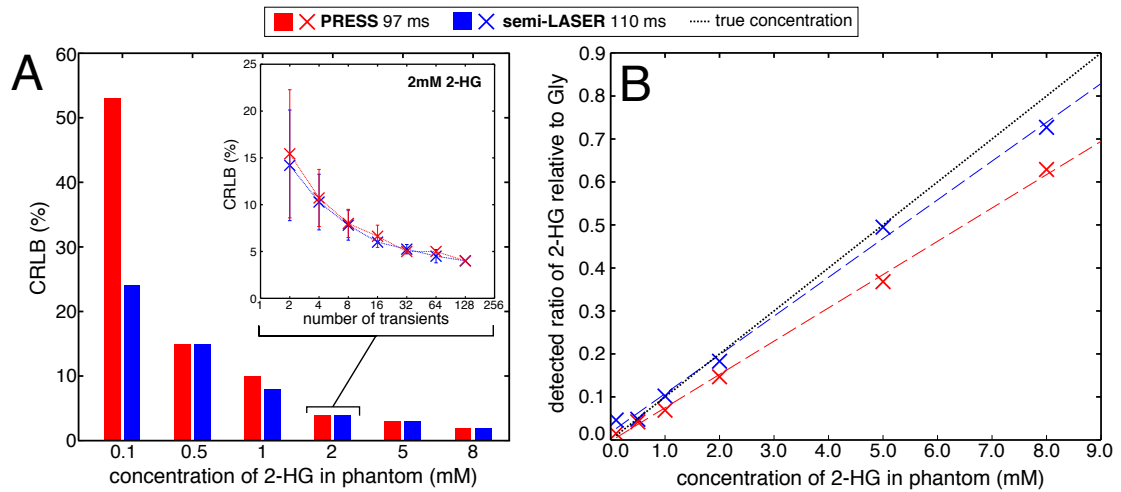


Figure 6.5: Sensitivity of semi-LASER and PRESS for 2-HG detection in phantom. **A:** Cramér–Rao lower bound (CRLB) of LCMoel fitting as a function of 2-HG concentration collected for 128 transients. Both methods show comparable errors across all concentration values. In addition, CRLBs shown as a function of number of acquired averages in a phantom bottle containing 2mM 2-HG. **B:** Ratio of detected 2-HG to Gly plotted as function of concentration compared to true concentration. Absolute values are not reported, due to T_2 decay time differences in phantom.

6.6 Detection of 2-HG with semi-LASER *in vivo*

The optimised method was then compared with the existing PRESS localisation *in vivo* in glioma patients. A total of 11 glioma patients (7 male; 4 female; age: 39 ± 11 years) were scanned with the optimised semi-LASER (TE = 110 ms) sequence in addition to PRESS (TE = 97ms). Of those, 6 patients were scanned with semi-LASER only, owing to constraints during a time-sensitive MR-protocol. Written and informed consent

ID	Sex/Age	Tumour/WHO Grade	IDH	PRESS		semi-LASER	
				conc. <i>mM</i>	CRLB %	conc. <i>mM</i>	CRLB %
P01	M/53	Glioblastoma/IV	WT	NA		1.25	40
P02	F/37	Glioblastoma/IV	WT	0.00	999	0.88	34
P03	M/45	Oligodendroglioma/III	WT	1.55	87	1.17	115
P04	M/52	Astroglial Tumour/III	IDH1	NA		3.87	15
P05	M/33	Anaplastic Astrocytoma/III	IDH1	NA		0.59	68
P06	M/22	Diffuse Astrocytoma/II	IDH1	2.74	22	1.95	21
P07	F/26	Oligodendroglioma/II	IDH2	NA		4.46	8
P08	F/56	Astrocytoma/III	IDH1 ^p	0.78	45	1.78	15
P09	F/44	Anaplastic Astrocytoma/III	IDH1	1.63	69	1.94	16
P10	M/24	TBC	TBC	3.81	13	2.91	11
P11	M/36	Diffuse Astrocytoma/III	IDH1	6.06	8	6.33	8
C01	F/30	-	-	0	999	0.32	112
C02	M/35	-	-	0	999	0.43	103
C03	F/24	-	-	0	999	0.66	59
C04	F/25	-	-	0	999	0	999
C05	M/24	-	-	0	999	0.14	228

Table 6.2: Summary of *in vivo* data for 11 glioma patients and 5 healthy controls with 2-HG concentrations (mM) and CRLBs including PRESS where obtained. TBC = To be confirmed. IDH1 or IDH2 indicates mutations in either enzyme. NA = Not Applicable (Data not acquired) and ^p denotes post-operative scan.

was given and approved by the South Central - Oxford B National Research Ethics Committee. Scanning was carried out prior to resection and histological confirmation, apart from one patient (P08) who underwent only a partial resection and whose residual tumour was scanned early post-operatively. In addition, 5 healthy volunteers (2 male; 3 female; age: 28 ± 4 years) were also scanned with both sequences. Voxels of $(20 \times 20 \times 20) \text{ mm}^3$ were acquired on 3T whole body MR Prisma/Trio systems and the protocol remained identical to the phantom investigation with 64–96 transients collected in patients and 96 in healthy volunteers. An unsuppressed water scan was also collected for absolute quantification.

6.6.1 Methods: Immunohistochemistry and DNA sequencing

After surgical resection, immunohistochemistry and DNA sequencing of the glioma samples was performed by S. J. Larkin to ascertain the mutation status of the IDH enzyme. Specific details regarding the procedures used can be found in Berrington

et al. [346]. The immunohistochemistry was performed on (4 μ m) tissue sections to detect the R132H IDH mutation and DNA sequencing was carried out on regions of IDH1 and IDH2 genes containing codons 132 and 172 respectively. Fig. 6.6 shows these immunohistochemistry and DNA-sequencing results from three patients.

6.6.2 Analysis

Basis sets are generated from the fully localised simulations to use in metabolite assignment with LCModel [315]. The basis set includes 2HG, Ala (alanine), Asc(ascorbate), Asp, GABA, Cr+PCr (total creatine: tCr), Gln, Glu, Gly (glycine), myo-Ins (myo-inositol), Lac (lactate), NAA+NAAG (total NAA: tNAA), PCho, PE, scyllo-Ins (scyllo-inositol), Tau (taurine), Glc (glucose), GSH (glutathione). Individual spectra were frequency- and eddy current corrected. For quantification of absolute metabolite concentrations, an exponential scaling correction, based on the transverse decay rate, T_2 , was applied to the unsuppressed water reference (assuming a bulk water concentration of 55.5 mM) using the average of recently published values in low and high grade tumours (150 ms) [363] and was set to 83 ms for healthy brain [364]. Because of the difficulties in measuring relaxation values of metabolites, a correction based on metabolite relaxation rates (T_1 and T_2) was not carried out in this work. All reported absolute concentrations are thus semi-quantitative in nature.

In order to assess the effect of overlaps on spectral fitting with LCModel, correlation coefficients are reported as the average of the pairwise correlation between individual metabolite fits X and Y , $r_{X:Y}$. These correlation coefficients are output by LCModel analysis for each spectral fit and act as an estimate of the dependence of fitting signal X on a particular metabolite signal Y . The LCModel manual states that if $r_{X:Y}$ is consistently less than -0.3 then the combined metabolite sum ($X + Y$) should be reported, since a large negative correlation implies that during the estimation of Y signal may be ‘stolen’ from X [315]. Genuine correlations of metabolite concentrations are, thus, not detectable by this pairwise fitting correlation and only signals which share mutual information via large overlap will result in large negative values of $r_{X:Y}$.

Signal-to-noise ratio (SNR) is reported as the LCModel output ratio of maximum signal height to $2\times$ the root mean square of the fitting residual. In this work, 95% confidence intervals ($\approx 2\times$ CRLB) are reported alongside the weighted mean of the absolute concentration values as described in the LCModel manual [315].

6.6.3 Results

The summary of the *in vivo* data for 2-HG detection is given in Table 6.2.

A visual comparison of the spectral quality achieved with both sequences was first made. Four examples of the *in vivo* spectral quality along with LCModel fits are shown in Fig. 6.7 - one example for each of the following: IDH1-mutant patient (P08); IDH wild-type (WT) (P03), to be confirmed (TBC) (P10) and healthy volunteer (Control) (C01). These spectra are representative of the high spectral quality obtained with both sequences. In particular, the amplitude of the residual water peak is less than tNAA or tCr, and no significant lipid contamination was observed. This resulted in flat baselines over the analysis range of 0.5–4.2 ppm except in one patient (P06), for whom spectral analysis was restricted to 3.8 ppm.

The presence of the H3–H3' multiplet can be seen clearly in the raw IDH1 tumour spectrum (Fig. 6.7) and in a further 6 patients (including the TBC-patient also shown in Fig. 6.7) scanned with semi-LASER. The occurrence of this peak at 1.9 ppm, upfield of the NAA singlet at 2.01 ppm, gives *in vivo* 2-HG semi-LASER spectra a distinctive spectral profile and can be seen in the raw data even before LCModel fitting.

In the 7 histologically-confirmed IDH-mutated tumours, semi-LASER CRLBs of 2-HG fitting $\leq 21\%$ were obtained in all but one patient (P05: 68%). For PRESS, only four datasets have been histologically confirmed as IDH-mutated. Due to this low number, a comparison using conventional CRLB rejection thresholds would introduce bias. Therefore, a comparison of the average 95% confidence interval for 2-HG in IDH-mutants was made on patients scanned with both methods ($n = 4$), finding concentrations of $(2.6 \pm 1.3)\text{mM}$ and $(2.3 \pm 0.7)\text{mM}$ for PRESS and semi-LASER, respectively. If patient P10 is included, whose IDH-status is yet to be confirmed,

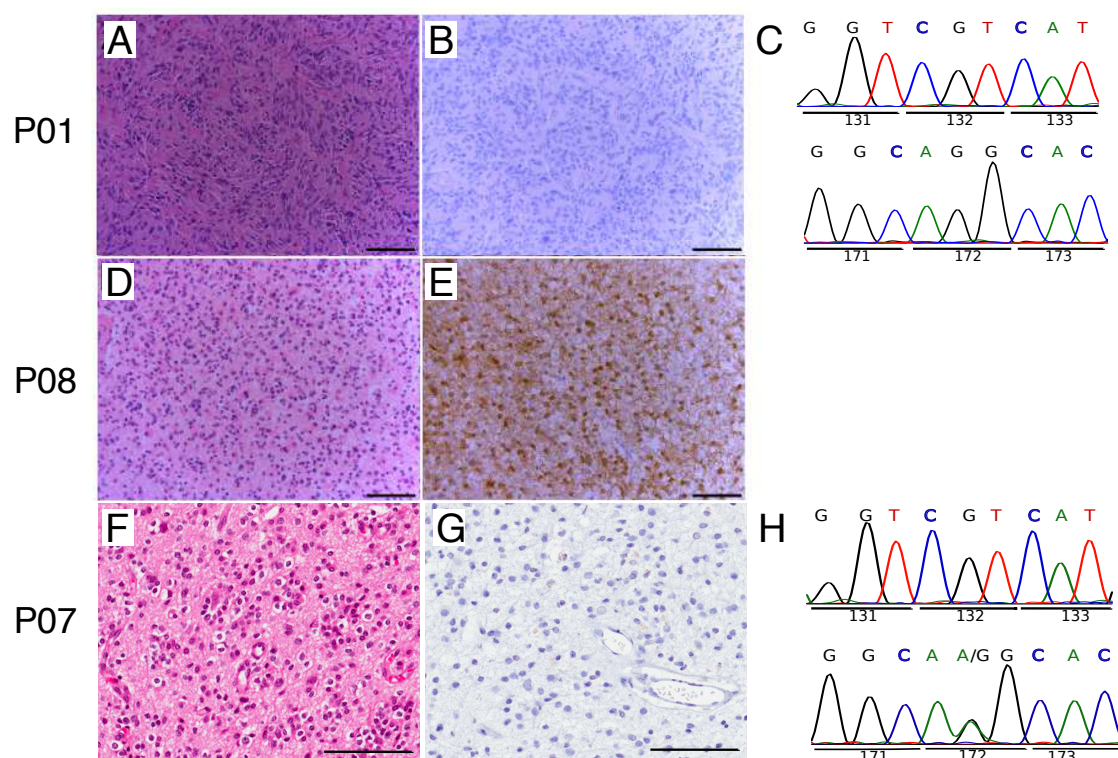


Figure 6.6: Tumour immunohistochemistry and sequencing as carried out by S. J. Larkin. Haematoxylin and eosin (A,D,F); immunohistochemistry using anti-IDH1 R132H antibody (brown reaction product) (B,E,G); PCR and direct sequencing of codon 132 of IDH1 (top trace) and R172 of IDH2 (bottom trace) (C,H). Case P01 (A,B,C): glioblastoma without IDH1 R132H mutation or IDH2 mutation; case P08 (D,E): astrocytoma with IDH1 R132H mutation; case P07 (F,G,H): oligodendroglioma without IDH1 R132H mutation but with IDH2 R172K mutation (P07 histological data previously published in Emir *et al.* [365]): All scale bars 100 μ m. Figures produced by S. J. Larkin.

these confidence limits become (2.8 ± 1.2) mM and (2.5 ± 0.7) mM for PRESS and semi-LASER, respectively. In both cases, detected concentrations are in agreement across methods yet confidence intervals are considerably improved using optimised semi-LASER. For the IDH-WT patients the minimum fitted CRLB is 34% for semi-LASER and 87% for PRESS. In all healthy controls 2-HG remained correctly undetected (CRLBs = 999%) using PRESS and also with semi-LASER all CRLBs > 100% except one (min: 59%, max: 999%). Patient (P10) (Fig. 6.7) who is awaiting biopsy, is thus predicted to be IDH-mutated, given the low CRLB (11%) using semi-LASER as well as the clear peak in the spectrum at 1.9 ppm.

Glu and Gln are also detectable with both methods, however Glu possesses higher CRLBs using semi-LASER than PRESS. Additionally, at 110 ms, the lactate peak at 1.33

ppm is almost fully inverted for semi-LASER, which significantly aids in its fitting generating much lower average CLRBs across all patients of $(10\pm2)\%$ compared to $(21\pm8)\%$ with PRESS.

Fig. 6.8 shows the average correlation coefficients across all metabolite fittings and patients for each sequence. It can clearly be seen that using both sequences the pairwise correlation coefficients between 2-HG and GABA are large (semi-LASER: $r_{2\text{HG:GABA}} = -0.55\pm0.26$; PRESS: $r_{2\text{HG:GABA}} = -0.53\pm0.14$) indicating a large degree of uncertainty and high signal overlap during fitting. Similar measures of the correlation coefficients for Glu are (semi-LASER: $r_{2\text{HG:Glu}} = 0.14\pm0.16$; PRESS: $r_{2\text{HG:Glu}} = 0.31\pm0.19$) and for Gln are (semi-LASER: $r_{2\text{HG:Gln}} = 0.01\pm0.09$; PRESS: $r_{2\text{HG:Gln}} = -0.02\pm0.06$).

6.7 Discussion

The aim of this work was to develop and characterise the use of a semi-LASER sequence for 2-HG detection at 3 T which is robust to chemical shift displacement artefact. The results reveal that a semi-LASER MRS sequence offers high sensitivity and localisation for *in vivo* detection of 2-HG, compared to an existing PRESS localisation scheme. The use of long-TE (110 ms) not only enables direct detection of the H3–H3' multiplet of 2-HG at 1.9 ppm in the *in vivo* spectra, but also generates reduced fitting correlations with overlapping glutamate and glutamine resonances and improved fitting of lactate compared to an existing long-TE PRESS sequence [345]. These improvements promise a more robust assignment of tumour metabolites for the investigation of tumour metabolism *in vivo*.

The need to consider the effect of localisation on the spectral pattern of the strongly coupled-spin system of 2-HG is strikingly demonstrated using 2-D simulations (Fig. 6.4). It has been shown that the loss of the H3–H3' multiplet with PRESS, as eluded to by Choi *et al.* [356], is due to a significantly large compartmental artefact within the voxel. This artefact is due to significant J-evolution under enhanced chemical shift displacement which arises from the narrow bandwidth RF pulses. This effect manifests as the formation of a large negatively phased signal component in the PRESS voxel, opposite to that expected using ideal pulses. This has a deleterious effect on any

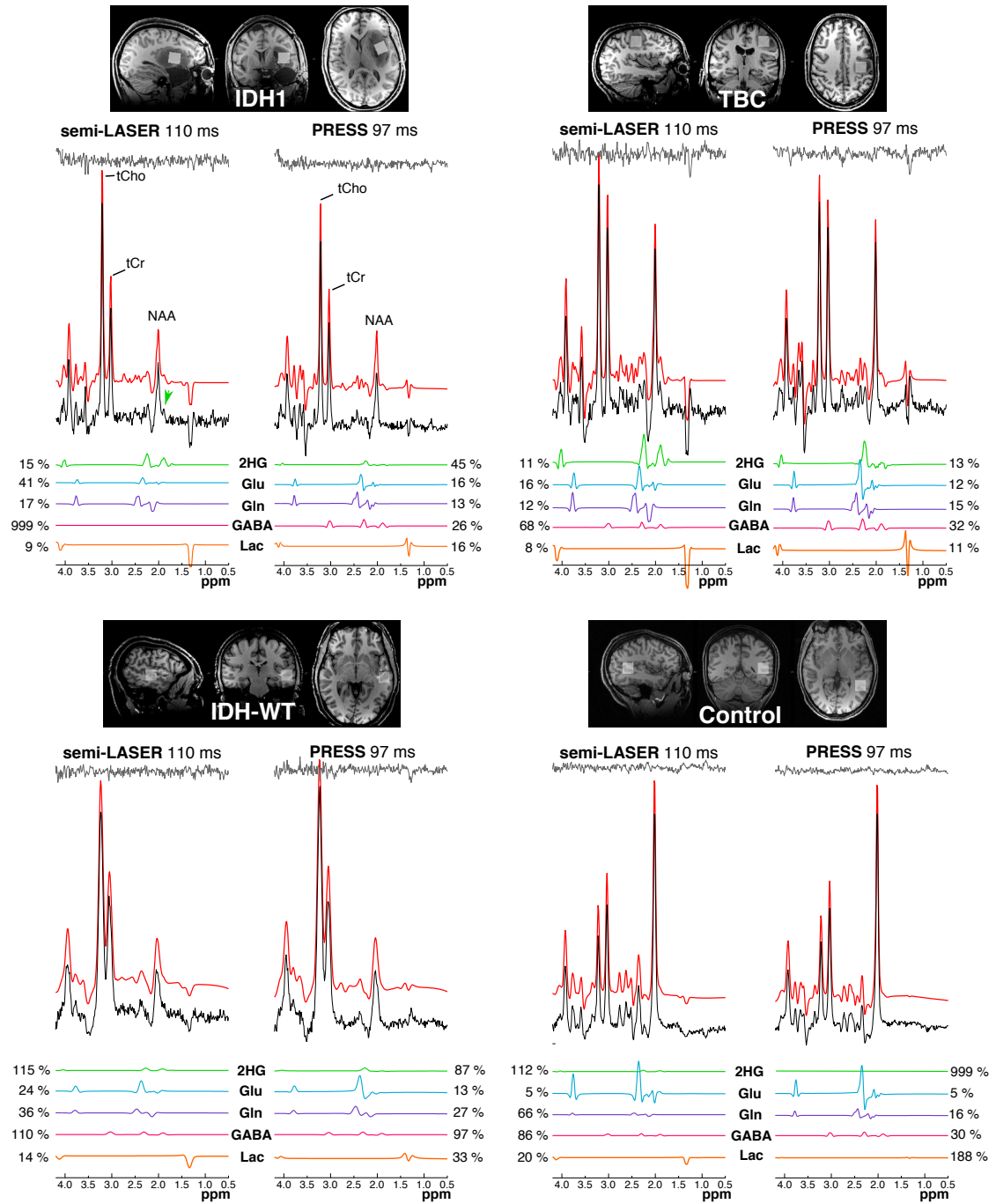


Figure 6.7: Example *in vivo* spectral acquisition using semi-LASER and PRESS. Data shown are from IDH-mutant (IDH1), To be confirmed (TBC), IDH wild-type (IDH-WT) and healthy volunteer (control). LCModel fits of 2-HG, glutamate, glutamine, GABA and Lac shown with CRLBs (%) given next to corresponding spectra. Green arrow in IDH1 indicates 2-HG peak visible with semi-LASER. Major peaks of NAA, total choline (tCho) and total creatine (tCr) are labelled on the IDH1 spectra for reference.

positive signal contribution from other compartments. The effect of signal cancellation using PRESS has been well documented for several spin-systems [46, 357] and this

may be worse for strongly coupled metabolites, of which coherence transfer and spin evolution become harder to predict [358]. Comparatively, semi-LASER suffers from considerably reduced compartmental artefacts, with an overall 2-HG spectral pattern very similar to that expected using ideal hard pulses (see Fig. 6.4). This finding is similar to that reported by Kaiser *et al.* [359], namely that using broadband adiabatic localisation on strongly coupled spins retains much of the ideal spectral pattern.

In this study, the chemical shift displacement across a single dimension (measured between the H3–H3' peak and the carrier frequency at 2.7 ppm) was 8.2% of the nominal voxel size using the Refoman6 pulses in PRESS and 1.9% using the 5.5 kHz HS4 R25 adiabatic pulse in semi-LASER. Even if the maximum B_1 requirement of semi-LASER is reduced to match PRESS, using longer duration pulses (10.2 ms, max B_1 = 13.4 μ T), the signal amplitude at 1.9 ppm is still around 12 times larger because of the increased bandwidth (2.46 kHz) of the broadband refocusing pulses. It is worth noting from a practical point of view that a strong chemical shift displacement artefact not only results in signal attenuation, as discussed above, but also metabolite measurement displaced from the prescribed voxel location, which is potentially problematic in the spatially heterogeneous environment of a tumour. Future work may endeavour to

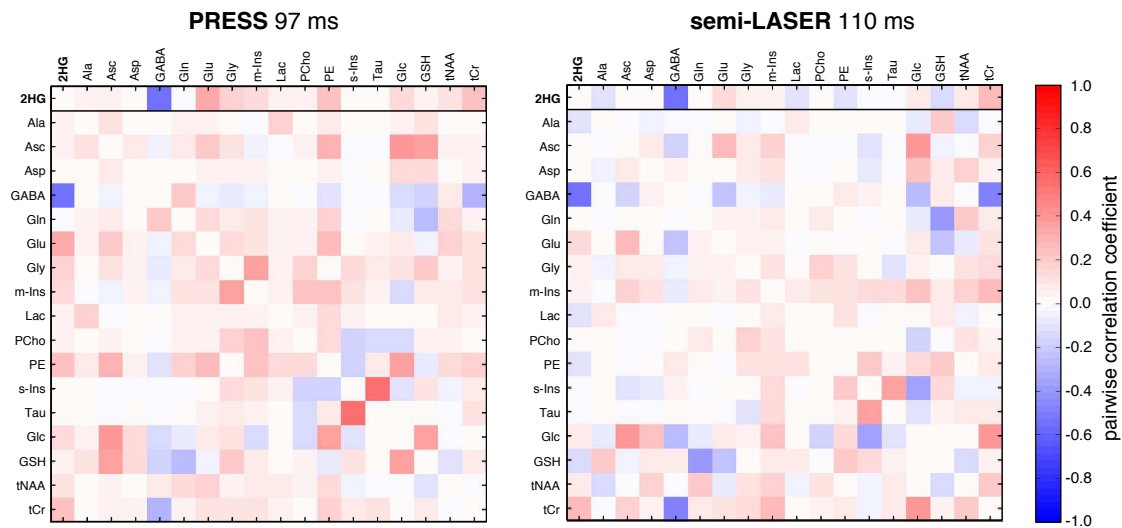


Figure 6.8: Average pairwise correlation matrices of LCModel metabolite fits across glioma patients showing PRESS (n = 7 patients) and semi-LASER (n = 11 patients). 2-HG is highlighted as the first entry in matrix.

improve the chemical shift displacement even further, by refocussing using FOCI [366] or GOIA [60] pulses, with the potential benefit of reducing total SAR [71].

The optimisation on the H3–H3' 2-HG, Gln and Glu overlaps resulted in an echo time that is 13 ms longer than PRESS. Considering J-modulation alone, this longer-TE resulted in H4–H4' (2.25 ppm) 2-HG peak amplitudes 29% smaller using semi-LASER than PRESS. This discrepancy did not appear to affect semi-LASER detection in the phantom. Indeed, CRLBs were similar for both sequences as a function of 2HG concentration and number of acquired averages. When comparing the detected 2HG to Gly ratios in the phantom to true values, semi-LASER appeared to detect more accurate concentrations than PRESS, although both methods tended to underestimate. Contrastingly, Choi *et al.* could correctly detect true phantom concentrations within the same range using the PRESS 97 ms sequence [356]. In the study presented here, only relative concentrations ratios in the phantom are reported, since a correction for T_2 relaxation was not carried out. T_2 relaxation values of 2-HG and Gly would account for the underestimation of concentration in both methods resulting from fitting on reduced signal amplitudes. The observation that concentrations were underestimated more using a shorter-TE PRESS sequence than with semi-LASER could be explained by the fact that during localisation with four refocusing pulses in semi-LASER, apparent signal decay from T_2 relaxation is somewhat reduced [367]. Micheali *et al.* have measured apparent T_2 values for NAA and Cr at 4T using CP-LASER and CP-PRESS and found values almost twice as large [73].

Although the H4–H4' (2.25 ppm) resonance is smaller in amplitude with semi-LASER, the presence of the H3–H3' resonance at 1.9 ppm helps detection. This is particularly true at very small concentrations of 0.1 mM, where the CRLB was 19% for semi-LASER and 53% for PRESS. Though not realistically detectable *in vivo*, detection at these low concentrations in the phantom indicate that semi-LASER may offer improvements on detectability at low concentrations of 2-HG. It is hoped that this sensitivity increase would benefit measurement from small tumour volumes, which have proven problematic for 2-HG detection in a recent study using PRESS (TE = 97 ms) [368].

In the *in vivo* acquisitions, the presence of the H3–H3' peak is clearly apparent alongside the NAA singlet in the IDH1-mutated patient shown in Fig. 6.7 (green arrow) and also in 6 other patients (5 IDH-mutant, 1 TBC). The identification of this peak in the raw spectra presents a clear benefit to rapidly ascertain IDH-status by obviating the need for more complex fitting algorithms. The proximity of H3–H3' to NAA at 2.01 ppm is 14.1 Hz at 3 T, thus if metabolite linewidths were around this order of magnitude or NAA concentrations were high (for example in lower grade gliomas [369]) this peak would become visually unresolvable. In all the patients in this study, however, reasonable linewidths were obtained and an inspection of the average pairwise fitting correlations between total NAA and 2-HG revealed very low average coefficients ($r_{2\text{HG};\text{tNAA}} = 0.09$) (see Fig. 6.8).

The narrower confidence interval on the estimation of 2-HG concentration in IDH-mutated tumours, using semi-LASER (2.3 ± 0.7) mM compared to PRESS (2.6 ± 1.3) mM, is also maintained after the inclusion of the patient with status yet to be confirmed. A direct comparison of semi-LASER and PRESS detection rates, using conventional CRLB rejection thresholds, was not appropriate in this study (due to the low number of patients). However, the use of 95% confidence limits, derived from CRLBs, did reveal that estimated 2-HG concentrations using semi-LASER were in agreement with those using PRESS and that semi-LASER yielded more consistent concentrations, as determined by the narrower confidence intervals. This optimised semi-LASER sequence may, therefore, offer an increase in reliability of 2-HG estimation, although investigation on a larger number of IDH patients is needed.

Correlational analysis revealed that on average the pairwise fitting correlation is larger for Glu using PRESS ($r_{2\text{HG};\text{Glu}} = 0.31$) than semi-LASER ($r_{2\text{HG};\text{Glu}} = 0.14$). A smaller pairwise correlation with Glu using semi-LASER is attributed to the additional 'degree of fitting' on the H3–H3' resonance where Glu overlap is reduced. However, CRLBs of fitting for Glu across patients and controls were higher for semi-LASER (29%) than for PRESS (18%). The reason for higher Glu CRLBs might be twofold. Firstly, accurate detection of low Glu concentrations using semi-LASER could result in larger CRLBs of detection. It is important to note that larger CRLBs do not necessarily equate to

inestimable metabolite concentrations and care should be taken to reject results with larger CRLBs as an inability to detect [370]. Interestingly, a recent *in vitro* study on the metabolic profile of IDH-mutants revealed decreases in Glu levels [284]. Secondly, a large 2-HG concentration, which lies on top of reduced Glu and Gln resonances at 1.9 ppm, could lead to an underestimation of these lower concentration metabolites. Further investigation in phantoms may help to understand the ability of the proposed method to determine Glu concentrations.

A limitation of this study is the reporting of absolute metabolite levels quantified using an internal water reference within tumour voxels. Factors that will affect this quantification method, such as regional differences in water concentration and metabolite relaxation in the heterogeneous tumour environment, are difficult to estimate in practice during patient studies. Apparent metabolite T_2 decay rates are variable with both tumour grade and MRS sequence used, and have the potential to complicate the quantification of metabolite concentrations [371]. There is a need for accurate measurement of T_2 relaxation rates of 2-HG and other metabolites using semi-LASER at 3T.

The low CRLBs ($\leq 40\%$) of 2-HG in two IDH wild-type tumours using semi-LASER indicate that this method may lack specificity in certain cases. The reason for these false positive assignments may be because of the failure of LCModel to fit GABA instead of 2-HG. Indeed, for one particular patient (P02; IDH-WT) 2-HG was fitted with a CRLB of 34%, yet GABA was not fit at all. The pairwise correlation coefficient of fitting between 2-HG and GABA is large and negative ($r_{2\text{HG:GABA}} = -0.55$) for semi-LASER suggesting that 2-HG and GABA are ‘stealing’ signal from each other. This could explain the lower average CRLBs for 2-HG in healthy volunteers using semi-LASER. It is worth noting that PRESS is also not immune to this effect given its correlation of ($r_{2\text{HG:GABA}} = -0.53$).

The unambiguous assignment of the H3–H3’ peak also relies on the fact that GABA concentration at this position is relatively small given its resonance at 1.89 ppm; 2-HG concentrations typically range from 5–35 mM in gliomas [264] therefore 2-HG should tend to dominate the spectrum. However, the presence of GABA in human gliomas is unclear. One *ex vivo* study of several tumour types did not report any GABA

detection [372], while a separate *ex vivo* study found GABA increases in low grade IDH mutated gliomas [373]. In the *in vivo* work done by Choi *et al.*, GABA was not detectable or existed at a very low levels across subjects [356]. In a recent study, using an optimised PRESS sequence at 7 T, Ganji *et al.* examined the effect of overlaps by removing 2-HG from the fitting basis, which resulted in a considerable influence on the detection of GABA along with Glu and Gln [374]. Removing GABA from the basis set may, however, lead to substandard metabolite fitting for early stage tumours or voxels containing healthy tissue.

To further address the issue of spectral contamination of 2-HG from the GABA resonance at 1.9 ppm, a further set of simulations and phantom studies were carried out. Figure 6.9 shows TE-optimisation simulations (as previously mentioned in section 6.3) repeated for the GABA spin-system. The chosen echo time of 110 ms (dotted

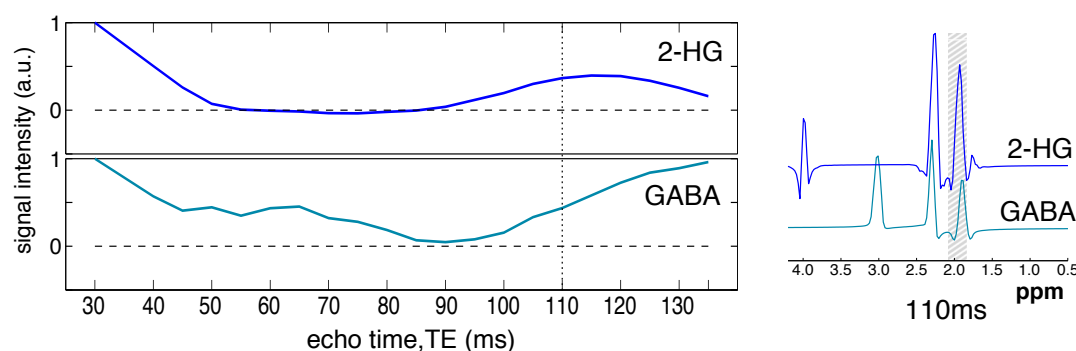


Figure 6.9: Effect of the optimised 110 ms echo time of semi-LASER on spectral overlap between 2-HG and GABA resonances. **Left:** Signal integral at 1.9 ppm is plotted as a function of echo time (TE) for 2-HG and GABA **Right:** Spectral profile at the optimised echo time of 110 ms for both spin-systems. Clearly there is a large degree of overlap between 2-HG and GABA at this echo time. Although other echo times are equally suboptimal with this scheme for removal of GABA overlap

line in Fig. 6.9 *left*) is clearly sub-optimal with respect to reducing overlapping GABA contribution. This can be seen directly in Fig. 6.9 *right* which shows the resulting spectra at 110 ms for both of these spin-systems. It is apparent that the spectral pattern of 2-HG at this echo time for multiplets of H3–H3' and H4–H4' is very closely matched by equivalent resonances of GABA arising from its H3–H4 and H5–H6 spins [375] and these are both upright and strongly overlapping. This H3–H4 resonance of GABA reaches a minimum signal intensity at 90 ms, which is around 11% lower than the

chosen TE of 110 ms. Furthermore, contributions from Glu and Gln are also nulled at this echo time (see Fig. 6.3). However, the 2-HG system is observed to have a similar signal evolution to GABA as a function of echo time and as such its signal integral is also reduced by around 10%, close to zero, at this echo time. Thus, also rendering 90 ms semi-LASER a suboptimal choice. It would, therefore, be an interesting avenue for future research to use GABA-specific MRS sequences to assess concentrations of this metabolite *in vivo*, in order to assess the requirement for further development in reducing GABA overlap *in vivo*.

A significant benefit of the 110 ms optimised echo time of semi-LASER is that lactate was quantifiable across all patients and controls with significantly reduced CRLBs compared to PRESS ($p=0.038$, paired t-test). At this echo time the lactate doublet at 1.3 ppm is almost fully inverted ($1/J = 144$ ms [376]), which allowed accurate estimation with semi-LASER. Importantly, the acquisition of lactate along with 2-HG is an interesting prospect because of its role in tumour metabolism [377]. Similar to the recent observations at 7T [365], elevated lactate levels were detected across all tumour patients compared to controls (2.3x, $p=0.003$, paired t-test).

More sensitivity and reliability for the detection of 2-HG, as well as other tumour metabolites such as glycine and glutathione, may be achieved at ultra-high-field (≥ 7 T) [354, 365, 374, 378], where gains from spectral resolution reduce problems with overlap. The detection of multiple metabolites, along with accurate estimation of 2-HG, could help build a more integrated picture of cellular metabolic processes *in vivo* and lead to further stratification of patients. For 3 T semi-LASER at 110 ms, wider cross-centre studies are required on larger patient groups to fully assess its ability to achieve this goal.

6.8 Conclusion

An optimised semi-LASER sequence at 3 T with long TE (110 ms) was shown to be capable of robust 2-HG detection in IDH-patients. Due to its reduced chemical shift displacement artefact compared to an existing PRESS localisation scheme, compartmental artefacts were considerably reduced, leading to the complete refocusing of

a resonance at 1.9 ppm. This distinct peak was clearly observable in the *in vivo* spectrum and aided fitting of 2-HG resulting in reduced confidence intervals of fitting. In addition, there was reduced Glu and Gln overlaps from the optimisation, which aided in the assignment of 2-HG. Lactate was also easier to detect with this method compared to PRESS owing to the slightly longer echo time. Since novel therapeutic approaches to glioma treatment are currently being developed, such as the targeted inhibition of IDH-mutants [296], it is anticipated that this method will be hugely beneficial for accurate *in vivo* assessment of tumour status and glioma progression at 3T through robust 2-HG and lactate estimation.

MRS of glioma at 7 T and 3 T

In this chapter, the benefits of ultra-high field (7 T) MRS acquisition of 2-HG signal in glioma patients is assessed. This is achieved through comparison of an existing semi-LASER sequence at 7 T with the method at 3 T previously demonstrated in Chapter 6. Firstly phantom studies were conducted to compare the sensitivity of detection at 3 T and 7 T. Spectral fitting of 2-HG was then directly compared *in vivo* in eight glioma patients (six carrying IDH-mutation). The sensitivity of detection was then assessed *in vivo* and improvement in fitting errors for 2-HG and other metabolites together with the spectral quality were compared. The two field strengths were also compared for the measurement of other metabolites in IDH-mutated glioma. Finally, the potential of ultra-high field for metabolomic analysis *in vivo* in glioma patients is explored.

7.1 Introduction

Recently, the World Health Organization (WHO) published updated guidelines for the classification of brain tumours, which, for the first time, incorporate genetic and molecular information [289]. One of the most commonly observed subtypes of glioma is characterised by mutations in isocitrate dehydrogenase (IDH) encoding genes and is found in ~80% of primary and secondary glioblastoma [243–245]. IDH-mutations affect two forms of the IDH enzyme residing in the cytosol (IDH1) and mitochondria (IDH2). Neomorphic activity of IDH-mutated enzymes leads to production and subsequent accumulation of the metabolite 2-hydroxyglutarate (2-HG) [264], which has been shown to be detectable with MR spectroscopy [351]. Robust and reliable detection of 2-HG in glioma patients, therefore, would provide a useful diagnostic tool for the stratification of individual brain tumour patients.

IDH-mutants undergo significant metabolic reprogramming, not limited to 2-HG production, to maintain energetic flux through the disrupted TCA cycle [264, 280,

287] (Fig. 7.1). In particular, IDH-mutants have been shown to upregulate genes (GLUD1/GLUD2) associated with glutaminolysis [279, 284], while certain enzymes (LHDA, PDH) associated with the glycolytic pathway show a reduced activity [11, 286]. IDH-mutants have also been associated with a low levels of the antioxidant glutathione (GSH)[379]. ^1H -MRS is able to detect concentrations of several key metabolites such as lactate, glutamate, glutamine and GSH *in vivo* and, hence, has the potential to provide a more integrated picture of the altered metabolism of IDH-mutants *in vivo* [12].

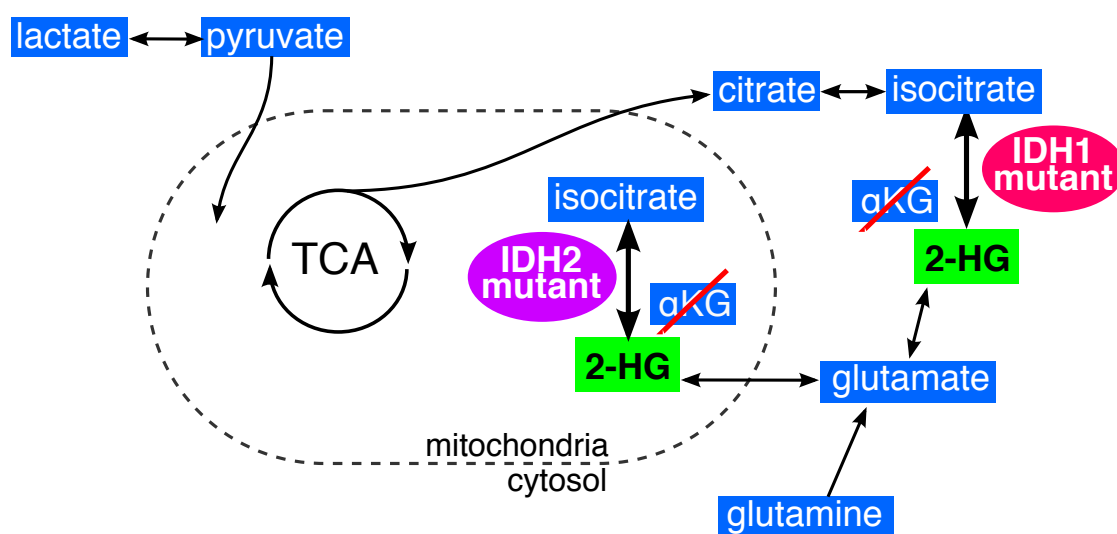


Figure 7.1: Schematic of some of the key metabolic pathways that are altered in IDH1 and IDH2 mutations. Metabolites detectable with ^1H -MRS, such as lactate, glutamate and glutamine, are potentially altered in the metabolically reprogrammed IDH-mutants.

The detection of 2-HG in brain tumours has been demonstrated *in vivo* at 3 T using short-TE [352] and long-TE [345, 356] PRESS sequences, in addition to spectral editing and 2-D correlation spectroscopy [380]. Detection of 2-HG is, however, hindered by signal overlap with neighbouring glutamate (Glu), glutamine (Gln), γ -aminobutyric acid (GABA) and glutathione (GSH) resonances. An optimised semi-LASER sequence described in this thesis (Ch. 6) was shown to improve 2-HG detectability at 3 T amongst these overlapping resonances through the improved refocussing of the H3-H3' (1.9 ppm) resonance [346].

7.1.1 Ultra-high field MRS

MRS at ultra-high field (≥ 7 T) benefits from an increased intrinsic signal-to-noise ratio ($\text{SNR} \propto B_0$) [47] and spectral resolution, due to the increased chemical shift dispersion ($\omega \propto B_0$). However, metabolite linewidths also increase due to shorter T_2 -decay times and increased susceptibility gradients [50]. SNR improvements at higher fields can thus fall below theoretical predictions [53], unless adequate localised shimming is achieved [54, 55]. Otazo *et al.* found a linear dependence on SNR for singlets of NAA, Cr and Cho [381]. Tkáč *et al.* reported increases in SNR above theoretical limits when comparing 7 T to 4 T, attributing this to a reduced receive coil penetration at 7 T [49]. Further studies have also observed at least linear increases in SNR with increasing field strength [55, 382]. The detection of a particular metabolite is, however, often determined by criteria based on Cramér-Rao Lower Bounds (CRLBs) of fitting [315, 370]. Theoretical analysis predicts that $\text{CRLB} \propto \sqrt{B_0}$ for singlet resonances [383] provided linewidths increase linearly with field strength. However, larger gains have been reported for J-coupled spin-systems *in vivo*, such as GABA, Gln and Glu [384], owing to decreased overlap of crowded multiplet resonances. For the strongly coupled metabolite 2-HG, 7 T MRS may, therefore, lead to significantly improved sensitivity and reliability of detection. In addition, MRS at 7 T can also achieve reliable detection of many other metabolites (e.g. Lac, Ins and GABA) [385] and can resolve nearby Glu and Gln resonances (≥ 4 T) [54] which may allow the investigation of wider metabolic reprogramming in IDH-mutants.

Bisdas *et al.* have used a STEAM acquisition at 9.4 T to acquire 2-HG *in vivo* in glioma patients [354]. The sensitivity increase at this field strength allowed them to measure GSH decreases in IDH-mutants, however chemical shift displacement was large (8 mm/ppm). Recently, Ganji *et al.* have optimised a long-TE PRESS scheme at 7 T for the detection of 2-HG *in vivo*, which generated smaller CRLBs ($\sim 5\%$) for 2-HG and reduced metabolite overlaps with GABA [374]. They also report significant variability in the spectral pattern of 2-HG for small variations in pulse bandwidth. Therefore, challenges of ultra-high field acquisition, from larger chemical shift displacements and also increased field homogeneity [57], present an issue for *in vivo* 2-HG measurement. Emir *et al.* have optimised a semi-LASER scheme for

detection of the 2.25 ppm resonance of 2-HG at 7 T [365]. With this method, they were able to separate individual patients with IDH1 and IDH2 mutated tumours using a spectral classifier. The method, with optimised TE = 110ms, was also able to reliably detect metabolites including Lac, Glu and GSH.

The increasing availability of human 7 T systems, together with a potential for large improvements in the accurate detection of 2-HG and associated metabolites, presents a unique opportunity for *in vivo* MRS in glioma patients. This study, therefore, aims to perform a direct comparison of detectability and sensitivity using existing long-TE semi-LASER localisation schemes at 3 T and 7 T. A comparison is, therefore, performed in phantoms and also by *in vivo* acquisition in eight glioma patients.

7.1.2 semi-LASER at 7 T

Fig. 7.2A shows the pulse sequence diagram for the semi-LASER sequence optimised at 7 T by Emir *et al.* [365]. Unlike that which was optimised at 3 T [378], this implementation of semi-LASER is symmetric in its positioning of refocusing pulses. The total echo time of the sequence (TE) is a combination of sub-echoes TE₁, TE₂ and TE₃. Prior to localisation, outer volume suppression (OVS) and water suppression (VAPOR) modules are included [301]. The two pairs of adiabatic refocussing pulses have broadband frequency range (bandwidth = 6 kHz) thus minimising the chemical shift displacement across a single dimension to a calculated value of 1 mm/ppm at 7 T for a 20 mm voxel size. For comparison, a recently optimised PRESS sequence [374] at 7 T has an equivalent displacement of 4.3 mm/ppm. Emir *et al.* optimised the sub-echo times for the 2.25 ppm resonance of 2-HG and found that a total echo time of TE = 110 ms generated a fully inverted absorptive lineshape at the 2.25 ppm (H4-H4') resonance and also an inverted resonance of lactate (Lac) at 1.35 ppm [365]. This echo time is coincidentally identical to the echo time optimised in Chapter 6 for detection of 2-HG at 3 T. The signal intensity of the H4-H4' peak as a function of the three sub-echoes is reproduced in Fig. 7.2B, with permission from the author.

Using custom built 2-D density matrix simulations written in Matlab (Mathworks, Inc, Natick MA) previously described in Chapter 6 of this thesis, the 2-HG spin system

was simulated in this work following localisation with the semi-LASER 7 T sequence. The spectral profile was compared to that of a pulse acquire experiment ($TE = 0$). The signal amplitude of the optimised (H4-H4') resonance was calculated to be 77% using the sequence of Emir *et al.* compared to the pulse acquire experiment, although this may be considerably more in practice because of T_2 relaxation (Fig. 7.2C). In contrast, Ganji *et al.* report a 56% signal amplitude relative to pulse acquire with their optimised PRESS method ($TE = 78$ ms).

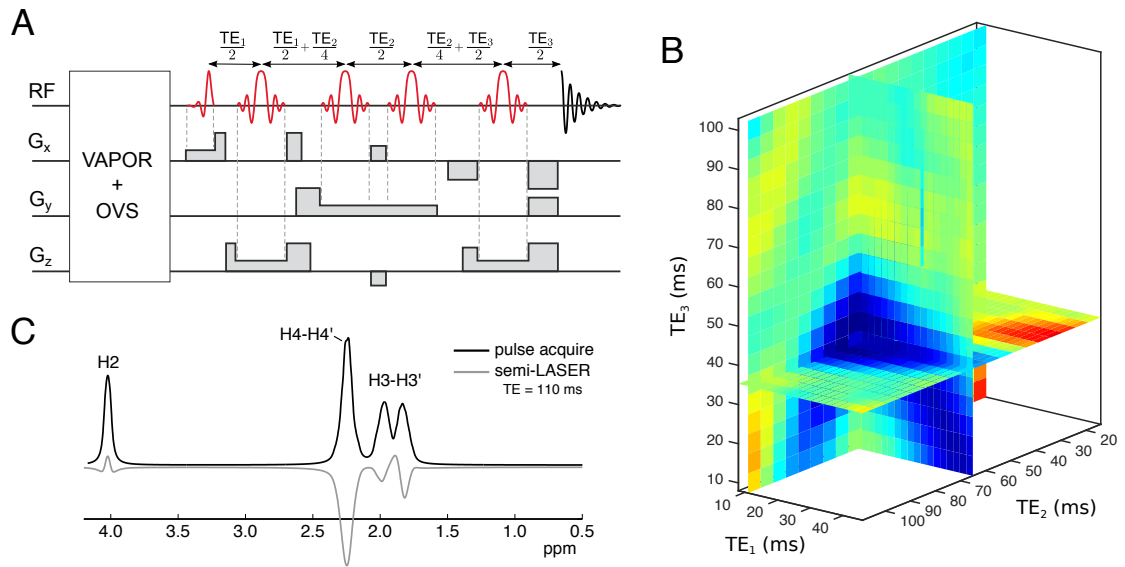


Figure 7.2: **A:** Pulse sequence diagram of the symmetric semi-LASER implementation at 7 T. **B:** TE optimisation on the sub-echoes TE_1 , TE_2 and TE_3 for the 2.25 ppm H4-H4' resonance of 2-HG. **C:** Comparison of the 2-HG spectral profile following the 110 ms semi-LASER sequence (grey) at 7 T and a pulse-acquire experiment (black). Spectra from 2-D density matrix simulations, spectra are line-broadened by 9 Hz. The signal amplitude of the H4-H4' resonance following semi-LASER localisation ($TE = 110$ ms) is 77% compared with pulse-acquire. Images **A** and **B** reproduced from Emir *et al.* [365] with permission from the author.

7.2 Aim

The primary aim of this study was to compare the benefit that long-TE semi-LASER detection of 2-HG has at 7 T compared to 3 T *in vivo*. In particular to assess the clinical benefit that 7 T may yield for 2-HG detection. To perform this comparison, the previously optimised sequence demonstrated at 3 T (Ch. 6) [378] was compared to a sequence at 7 T [365]. Identical echo times and localisation schemes reduce the

variability between field strengths. Factors that may differ between field strengths, such as relaxation times, and differences in coil sensitivity, are difficult to control for in a clinical comparison of this study. Thus, they form part of the discussion at the end of this chapter.

7.3 Phantom Studies

7.3.1 Methods

To assess how the sensitivity increase at 7 T influences the detection of 2-HG, a multi-compartment phantom was constructed with varying 2-HG concentrations similar to that described in Chapter 6. Six 66 ml bottles containing 2-HG (R,S- α -hydroxyglutaric acid, Sigma-Aldrich) were constructed at measured concentrations of 0.1, 0.5, 1, 2, 5 and 8 mM in distilled water, each with 10 mM of glycine (Gly) for reference. Phantom solutions also contained a PBS buffer solution and the average measured pH = 7.5 ± 0.1 . All six bottles were scanned over two acquisitions since only five bottles could be housed in the larger cylindrical container of the phantom. Fig. 7.3A shows coronal and transverse T_1 -weighted image slices of the phantom acquired at 7 T.

A total of 128 averages were acquired for each of the concentrations apart from the smallest concentration of 0.1 mM, for which 256 averages were acquired. Acquisition parameters for both field strengths were as follows: TE = 110 ms, spectral width = 6 kHz, number of samples = 2048 and voxel size = $(20 \times 20 \times 20)$ mm³. Repetition times (TR) were kept long to avoid T_1 weighting of spectra; TR = 3 s and TR = 5.85 s at 3 T and 7 T, respectively. Adiabatic inversion was achieved using the same HS4 R25 refocusing pulses at 3 T (bandwidth = 5.5 kHz, duration = 4.5 ms, peak B_1 = 28 μ T) and 7 T (bandwidth = 4.1 kHz, duration = 6 ms, peak B_1 = 19 μ T). First and second order shims were calculated using gradient echo shimming at both field strengths [311] and first order shims were optimised using FASTMAP acquisition at 7 T [312]. Water linewidths as measured on the scanner were found to be ≤ 3 Hz. A B_1 calibration was also performed on the 90° excitation to ensure sufficient transmit power in each voxel. Spectra were analysed using LCModel [315] after applying B_0 and first-order phase

corrections. The concentration of 2-HG was measured relative to the concentration of detected glycine in the spectra.

7.3.2 Removal of residual water

Compared to 3 T, finding optimum localised shim solutions in the phantom at 7 T was challenging. The inhomogeneous static magnetic field distribution combined with a subsequent requirement for large second order shim terms resulted in spurious refocusing of water in some of the acquired spectra. This spurious refocusing is highlighted in Fig. 7.3B (grey line), plotted over the spectral range (0.5–5.5) ppm, where the sub-optimal shimming manifested as a large baseline artefact. This was therefore corrected in post-processing by applying a Hankel Lanczos singular value decomposition (HLSVD) on the spectra centred on the water peak at 4.7 ppm. A model order ($K = 30$ exponentially damped sinusoids) was selected empirically and was similar to a previously published optimum value for water peak removal in ^1H -MRS [386]. The HLSVD removal of the water peak was applied to the spectra at 7 T and the result can be seen as the black line in Fig 7.3B (8 mM 2-HG , $N = 128$).

The resulting spectra were fit with LCModel using a basis set containing 2-HG and Gly, generated from full 2-D density matrix simulations. Fig. 7.3C shows the accuracy of the simulated basis sets to capture the spectral information for the bottle containing 8 mM 2-HG at 3T (top) and 7T (bottom). Spectra were line-broadened by multiplication of the FID with decaying exponential to match *in vivo* linewidths of 6 Hz at 3 T and 14 Hz at 7 T. The semi-LASER sequence at 3 T, which is optimised for the 1.9 ppm resonance generates upright peaks for all peaks of 2-HG. At 7 T ($TE = 110$ ms) the 2.25 ppm peak is inverted. In both cases, the generated basis sets fully captured the spectral profiles of 2-HG.

7.3.3 Sensitivity of Detection

An analysis of the detection of 2-HG at both field strengths was then conducted in the phantom. For each increment in 2-HG concentration, binned spectra were generated post-acquisition containing 2^n transients where $n = 1, 2 \dots 7$ (except for 0.1

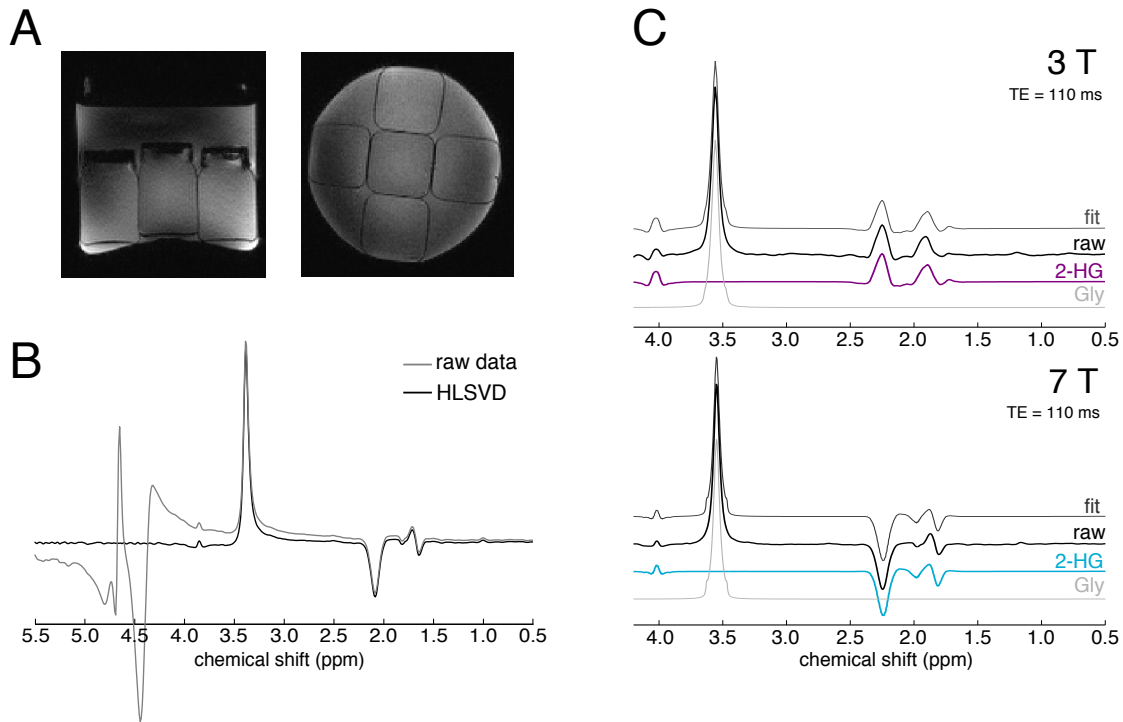


Figure 7.3: **A:** 2-HG sensitivity phantom containing 5 removable 66 ml bottles. Bottles contained 2-HG at concentrations of 0.1, 0.5, 1, 2, 5 and 8 mM, including 10 mM glycine. **B:** Raw spectrum (grey) from a 7 T acquisition (8 mM 2-HG, $N = 128$) showing large water contamination. After HLSVD this artefact is removed (black). **C:** LCMoel fits of the 8 mM 2-HG phantom for 3 T (top) and 7 T (below), showing the different spectral patterns.

mM where $2^8 = 256$ transients were collected). Each spectrum was fit using LCMoel and the Cramér-Rao lower bounds (CRLBs) of fitting along with the ratio of detected 2-HG to glycine were examined. These data are plotted in Fig. 7.4A. A CRLB of 40%, which is around the (30-50)% rejection thresholds commonly used *in vivo* [28, 370], is marked on each plot.

Across all concentrations, the fitting of the 2-HG signal is accompanied by a reduced CRLB at 7 T compared to 3 T as is expected from the increased SNR. For the intermediate concentrations (2 and 5 mM), $\log(\text{CRLB})$ (in Fig. 7.4) decreases roughly linearly with $\log_2(n)$, which may be expected if the CRLB is inversely proportional to the number of acquired averages, with some constant α , ($\text{CRLB} \propto n^{-\alpha}$). At 2 mM of 2-HG in phantom, only 8–16 spectral averages were needed at 7 T to match the CRLB achieved with 128 averages at 3 T, which is fewer than expected if CRLB purely reflected the theoretical increase in SNR ($NT = 24$). At 5 mM and 8 mM concentrations the

CRLB reaches a minimum value at both field strengths. In the bottles containing the smallest concentrations of 2-HG a transition in CRLB occurs following the acquisition of 64, 16 and 8 averages at 0.1, 0.5 and 1 mM at 7 T and 128, 32 and 16 at 3 T. The rapid decrease in CRLBs may reflect the limits of detectability at either field strength, since CRLBs fall sharply from $\sim 100\%$, which is the upper limit of detection suggested in the LCModel manual [315]. Additionally, within the first few spectral averages, significant unwanted coherences were observed while scanning, which remain until phase-cycling is complete. These coherences may negatively influence fitting of small concentrations of 2-HG.

Fig. 7.4B compares the fitted concentration with 64 averages across all concentrations of 2-HG. Concentrations are reported relative to detected glycine and, thus, rely on the assumption of an accurate fit of this metabolite across all acquisitions. These concentration ratios are expressed as a percentage of the theoretical amount present in each bottle. A 5% error on the theoretical concentrations was assumed (grey highlighted region, Fig. 7.4B), which accounted for experimental error during the construction of the phantom and is equivalent to a previously suggested value [374].

At either field strength, the measured concentrations fell within this $\pm 5\%$ interval of the theoretical concentration. The only deviation from this was the 1 mM 2-HG concentration at 7 T, where 2-HG concentration was underestimated by around $(20 \pm 10)\%$. The reason for this discrepancy can be seen in Fig. 7.4C. The 2-HG resonance at 4.02 ppm was absent in both the fitted spectrum and the raw spectrum (pre- and post-HLSVD, inset figure). This was attributed to suboptimal shimming and a large water baseline artefact, which becomes the dominant signal contribution at the chemical shift of the H2 resonance of 2-HG. The absence of spectral information from this 4.02 ppm peak led to an underestimation of the total underlying 2-HG signal. The artefact was also present to a lesser extent at 0.5 mM of 2-HG (data not shown). At 0.1 mM, there was a slight overestimation of 2-HG concentration at both field strengths. This could be explained by over fitting 2-HG in a spectrum dominated by decreased signal-to-noise. Larger confidence intervals of quantification, however,

reflected this increased uncertainty. Confidence intervals are consistently lower across all concentrations at 7 T than 3 T, as is expected from Fig. 7.4A.

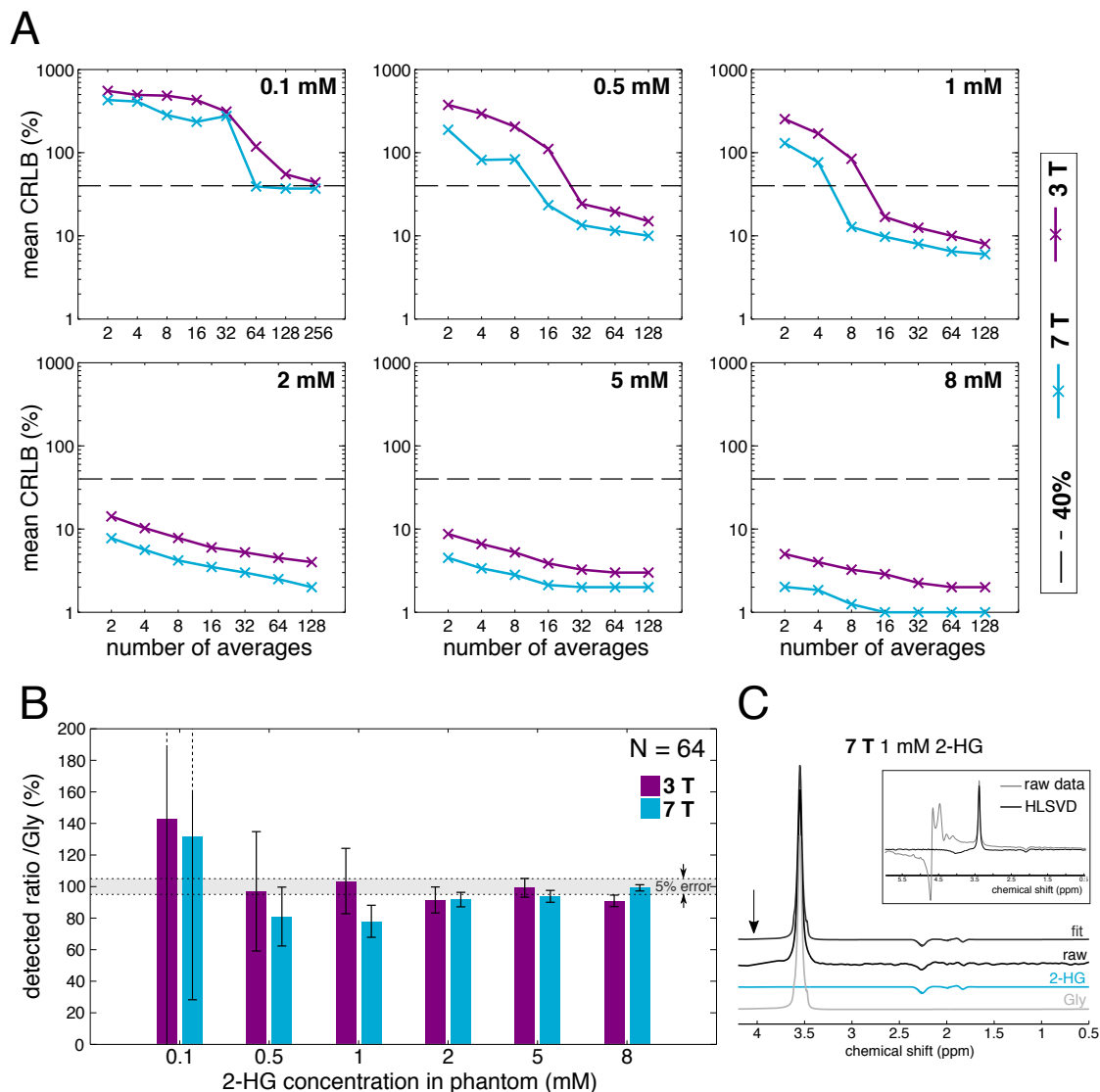


Figure 7.4: **A:** Mean CRLB as a function of the number of averages for each measured concentration of 2-HG for 3 T (purple) and 7 T (blue) in logarithmic scale. A 40% CRLB threshold is also shown (dotted line). **B:** LCModel detected ratio of 2-HG concentration relative to glycine (expressed as a percentage of the total) for all concentrations (N = 64). An experimental error of $\pm 5\%$ on the concentration of 2-HG in phantom is shown in grey. Error bars reflect approximate 95% confidence interval as estimated from CRLBs. **C:** LCModel fit for the 1 mM 2-HG bottle at 7 T showing the absence of the H2 peak at 4.02 ppm. This peak is missing even before HLSVD is applied (inset figure) owing to a large spurious echo at this chemical shift

7.4 *In vivo* comparison

7.4.1 Methods

A direct comparison of metabolite detection in IDH-mutated tumours at 3 T and 7 T is performed using semi-LASER localisation on eight tumour patients (5M/3F, Age = 42 ± 10 , $n = 8$). Written and informed consent was approved by Oxfordshire B National Research Ethics Committee. Some of the *in vivo* data acquired at 7 T has previously been published by Emir *et al.* [365]. In addition, five of the patients scanned for the previous Chapter (Ch. 6) at 3 T, were included in the subsequent analysis. In all cases, patients were scanned over two sessions at either field strength on whole body MR systems (Siemens, Erlangen), both equipped with 32-channel receive array head coils. After scanning, the majority of patients underwent surgical resection ($n = 7$). Immunohistochemistry and DNA sequencing of the tumour to determine the mutation status of *IDH1* and *IDH2*, was performed by S. J. Larkin, in an identical manner to that described in Section 6.6.1. One patient (P08) was still awaiting surgery and histological confirmation at the time of writing. A total of 6 patients were IDH-mutated (*IDH1*: $n = 5$; *IDH2*: $n = 1$) and a single patient was characterised as IDH-wildtype ($n = 1$). The summary of these data, in addition to the histological grading of each tumour and detected 2-HG concentrations can be seen in Table 7.1.

A $(20 \times 20 \times 20)$ mm³ voxel was positioned, with reference to a 1 mm isotropic T_1 -MPRAGE acquisition, in a tumour region of maximum homogeneity at each field strength. Care was taken to manually position the voxel in a similar position at 3 T and 7 T. The 2-HG optimised semi-LASER localisation schemes at 3 T and 7 T [365] ($TE = 110$ ms, $TR = 3 / 5$ -6 s, spectral width = 6 kHz, $np = 2048$, $NT = 71$ -128) were preceded by an outer volume suppression (OVS) module and VAPOR water suppression [301]. Pulse parameters were the same as the phantom acquisitions. Similar to the phantom acquisitions, first- and second-order shimming was performed with GRESHIM [311] and first-order shims were adjusted at 7 T using FASTMAP [312]. At 7 T, a high permittivity barium titanate ($BaTiO_3$) dielectric pad was positioned close to the subjects' head to increase efficiency of the transmit field B_1^+ in the voxels [59],

ID	Sex/Age	Tumour/WHO Grade	IDH	3 T			7 T		
				conc.		%	conc.		%
				Cr	Cho		Cr	Cho	
[†] P01	M/52	Astroglial Tumour / II	IDH1	6.9	1.7	15	8.4	1.6	5
[†] P02	M/53	Glioblastoma / IV	WT	2.8	0.9	40	0.6	0.2	64
[†] P03	F/26	Oligodendroglioma / II	IDH2	11.6	3.5	8	13.5	4.8	3
[†] P04	F/44	Anapl. Astrocytoma / III	IDH1	3.1	0.9	16	4.3	1.2	9
P05	M/34	Diff. Astrocytoma* / II	IDH1	9.3	1.4	11	8.0	1.5	5
P06	F/36	Diff. Astrocytoma / II	IDH1	5.7	1.6	23	7.6	1.7	6
[†] P07	M/36	Diff. Astrocytoma / III	IDH1	14.4	2.7	8	17.3	1.8	4
P08	M/52	TBC	TBC	7.6	1.8	9	7.9	1.7	5

Table 7.1: Summary of 2-HG detection using LCModel across 8 tumour patients using semi-LASER at 3 T and 7 T. Six patients were determined to have IDH mutation (IDH1, N = 5; IDH2, N = 1) and one patient (P02) was found to be IDH-wildtype (WT). The remaining patient (P08) is awaiting biopsy and histological confirmation (TBC). [†] denotes that the data was also included in the analysis of Chapter 6. * probable classification - too few cells for accurate histological confirmation. Concentrations of 2-HG relative to total creatine (Cr) and total choline (Cho) are reported for comparison alongside Cramér-Rao Lower Bounds (CLRBs) of fitting (%).

as was described by Emir *et al.* [365]. An unsuppressed water spectrum was acquired after spectroscopic acquisition for eddy-current correction. A total of 64–128 averages were acquired from each participant, subject to the time constraints of carrying out MRS in an already busy scanning protocol.

LCModel [315] was used to fit metabolite concentrations using fully-localised basis sets of 21 metabolites containing 2-HG, Ala, Asc, Asp, Cr, GABA, Gln, Glu, Gly, Ins, Lac, NAA, NAAG, PCho, PCr, PE, sIns, Tau, Glc, GPC and GSH generated from 2-D density matrix simulations with real refocussing pulse information Matlab (Mathworks, Inc., Natick, MA). Fitted concentrations are reported relative to an internal reference of either total creatine ($tCr = Cr + PCr$) or total choline ($tCho = PCho + GPC$). A correction for T_2 decay time differences between individual metabolites is not carried out in this work. The approximate 95% confidence intervals on metabolite concentrations are calculated as $\approx 2 \times CRLB$ [315]. Metabolites with CRLBs $\geq 100\%$ are considered undetected.

7.4.2 Spectral Comparison

A visual comparison of the spectral quality achieved *in vivo* at 3 T and 7 T was firstly carried out. Fig. 7.5 shows the spectra acquired at 3 T and 7 T with semi-LASER

localisation (TE = 110 ms) for four tumour patients. Voxel positioning is also shown for each patient revealing similar placement between acquisitions at 3 T and 7 T. Beneath each spectrum the LCModel fits of several overlapping metabolites of interest are shown, namely 2-HG, GABA, Glu, Gln, Gly, GSH, Ins and Lac. Spectra at both fields strengths were free from baseline artefacts or significant contamination from lipids or other coherences over the (0.5–4.2) ppm range, apart from one (P05), for whom macromolecular contamination meant spectral analysis was restricted to (1.5–4.2) ppm at both field strengths. *In vivo* acquisition at 7 T was less susceptible to the sub-optimal shimming observed in the phantom studies, noted by the absence of large residual water peaks, baseline artefact and the low residual signal.

The optimised spectral pattern of 2-HG is present in the raw spectra of the IDH-mutated patients with large visible inverted resonance at 2.25 ppm at 7 T and an upright resonance at 1.9 ppm at 3 T (Fig. 7.5). In addition, the corresponding spectral feature of the H3-H3' resonance (at 1.9 ppm) is also visible at 7 T and to a lesser extent similarly the H4-H4' (2.25 ppm) at 3 T, however this spectral peak overlaps with Glu and Gln. CRLBs of individual metabolite fits for the displayed *in vivo* spectra are also provided adjacent to spectral fits. The CRLB of fitting 2-HG in the single IDH-WT patient was higher at 7 T (64%) compared to 3 T (40%). The IDH-WT spectra also contained a large glycine (Gly) resonance (3.55 ppm) detected at 3 T (10%) and 7 T (7%). Glutathione (GSH), which is highly overlapped with Glu, Gln and 2-HG resonances, was quantified in all the spectra of Fig. 7.5 at 7 T, yet failed to be assigned at 3 T. The Lac doublet (1.31 ppm) appears near inverted using both semi-LASER sequences TE = 110 ms (for Lac, $1/J = 144$ ms). Concentrations of GABA were also undetected in all patients at 3 T, yet is detected in three further patients in total at 7 T.

7.4.3 Sensitivity *in vivo*

To examine the increase in 'sensitivity' for *in vivo* 2-HG detection at 7 T, the acquired spectra from each patient were split into averages containing NT = 1, 2, 4, 8, 16, 32, 64 acquisitions and then LCModel analysis was performed on these averaged spectra. The CRLBs of 2-HG detection were then obtained.

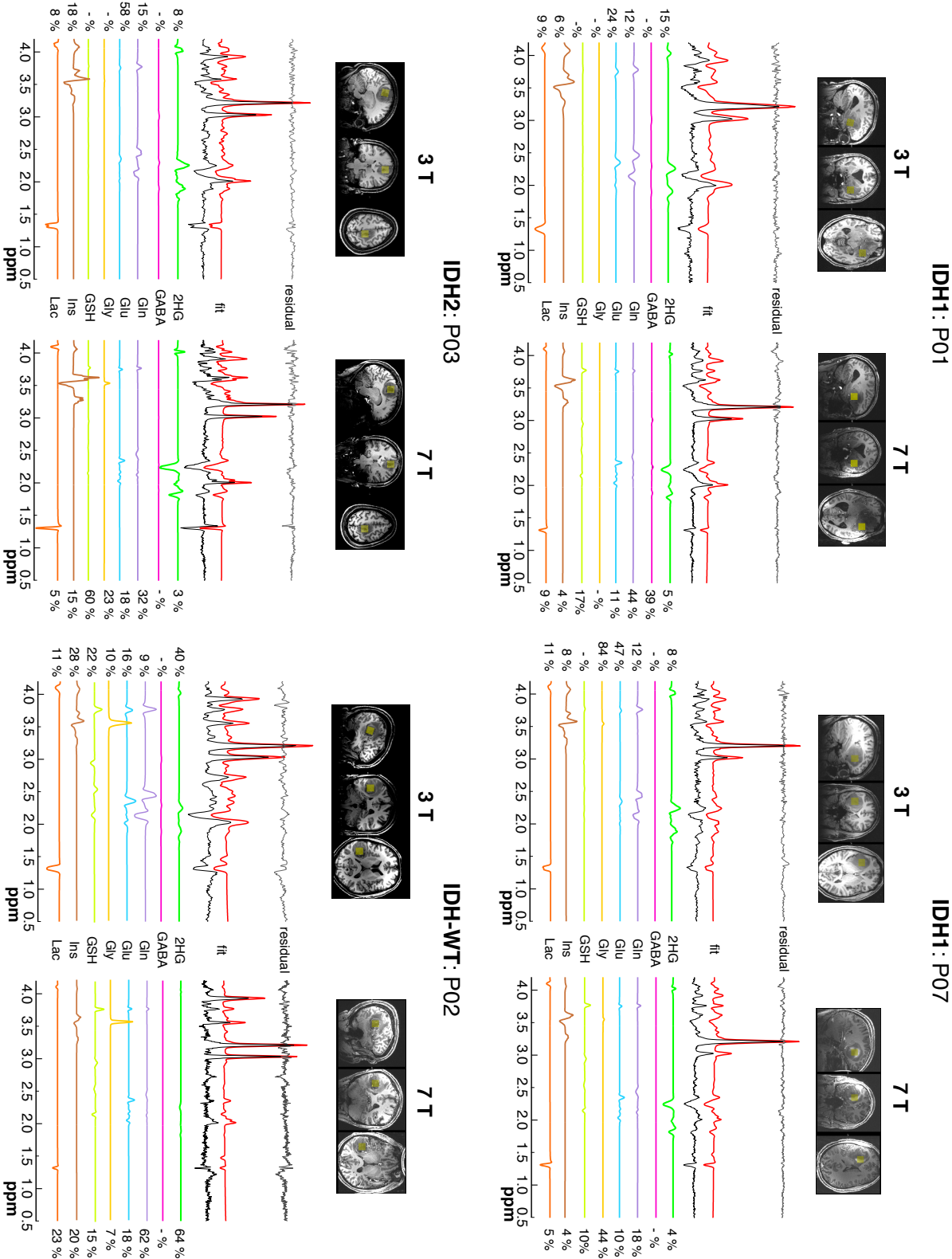


Figure 7.5: Example *in vivo* spectral acquisition using semi-LASER at 3 T and 7 T. Data are from IDH1-mutant, IDH2-mutant and IDH wild-type (IDH-WT). LCModel fits of 2-HG, glutamate, glutamine, GABA, Gly, GSH, Ins and Lac are shown with CRLBs (%) next to corresponding spectra. Voxel placement is also shown for each patient at both field strengths.

Fig. 7.6A shows spectra containing 2 to 64 averages from one patient (P03) at 3 T and 7 T. The dotted vertical lines indicate positions of the 2-HG resonances, H2 (4.02 ppm), H3-H3' (1.9 ppm) and H4-H4' (2.25 ppm). At 64 averages, the large concentration of 2-HG in this tumour is visible in the spectrum at H3-H3' and H4-H4' at both field strengths. At around 8 acquisitions the H3-H3' signal becomes distinctly less clear at 3 T. However, at 7 T the large inverted H4-H4' resonance is visible in the spectrum after only 2 spectral averages.

Fig. 7.6B shows the mean CRLB of 2-HG fitting averaged over five patients (individual transients not available for $n = 2$ patients) after LCModel fitting. Highlighted regions reflect standard error in the CRLB values. CRLBs are consistently lower at 7 T than 3 T, similar to the phantom sensitivity measurements. In fact, even with only a single acquisition the mean CRLB at 7 T is $(28 \pm 6)\%$, below common rejection thresholds of 30-50%. The CRLB then drops to $(20 \pm 5)\%$ after NT = 2. For 3 T, NT = 8 was found to lower the mean CRLB of fitting below 40% to $(38 \pm 11)\%$ and a CRLB of less than 20% was achieved with NT = 35. The boost in sensitivity for 2-HG detection to achieve a 20% CRLB of fitting at 7 T results in theoretical scan time reduction of 62%.

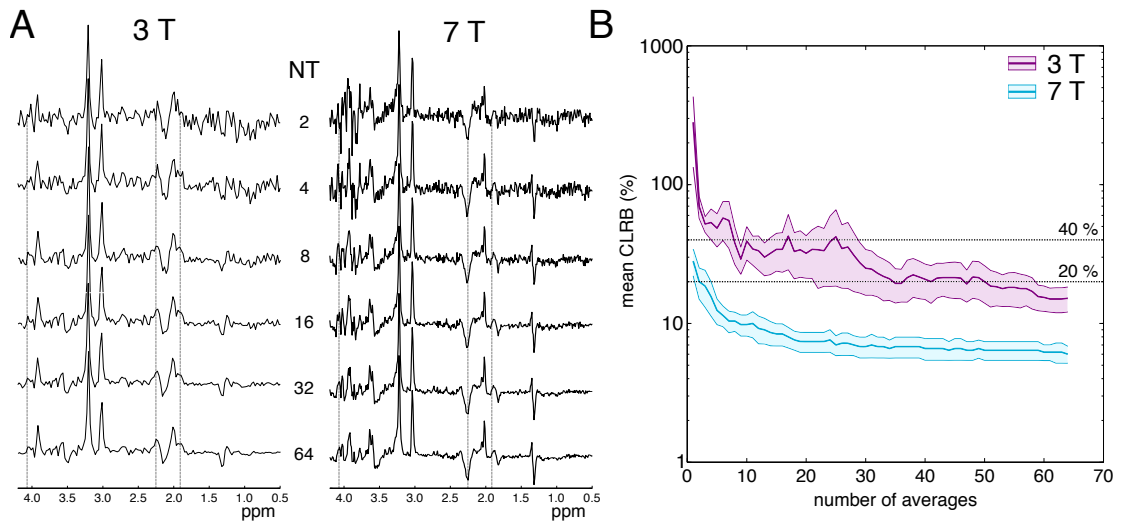


Figure 7.6: Comparison of the detection sensitivity *in vivo* using 3 T and 7 T. **A:** Raw spectra (patient P03, IDH2) shown with increasing number of averages 2–64. Vertical lines indicate chemical shift of 2-HG resonances. **B:** Mean CRLB of 2-HG detection 5 patients shown as a function of the number of averages. Standard error plotted as highlighted region. CRLB thresholds of 40% and 20% also shown.

7.4.4 Mean CRLBs and concentrations

The mean CRLBs of metabolite detection are compared across all IDH-mutated tumour spectra ($n = 6$) in Fig. 7.7. For each quantified metabolite, the CRLB is plotted for 3 T and 7 T with its standard deviation highlighted by the shaded region. 2-HG was detected across all IDH-mutated tumours and the CRLB of fitting at 7 T (5 ± 2)% was found to be significantly smaller than at 3 T (14 ± 5)% ($p = 0.009$, paired two-sided t-test). Glu and Lac showed trends for reduced CRLBs at 7 T although these were not significant with the current number of patients. Errors of fitting the *myo*-inositol (Ins) signal were significantly reduced ($p = 0.002$) at 7 T (2.3 ± 0.5)% compared to 3 T (4.2 ± 1.3)%. Glycine (Gly) was detected in 3 patients at 3 T and 4 patients at 7 T, where it also benefited from a trend of reduced CRLBs ($p = 0.20$). At 3 T, two metabolites were only detected in a small number of patients, or not at all, namely GABA ($n = 0$) and GSH ($n = 1$).

Interestingly, the difference in Glutamine (Gln) fitting was found to be significantly different between field strengths ($p = 0.024$), however a higher CRLB was found at 7 T (44 ± 25)% compared to 3 T (18 ± 7)%, which is discussed later in this chapter.

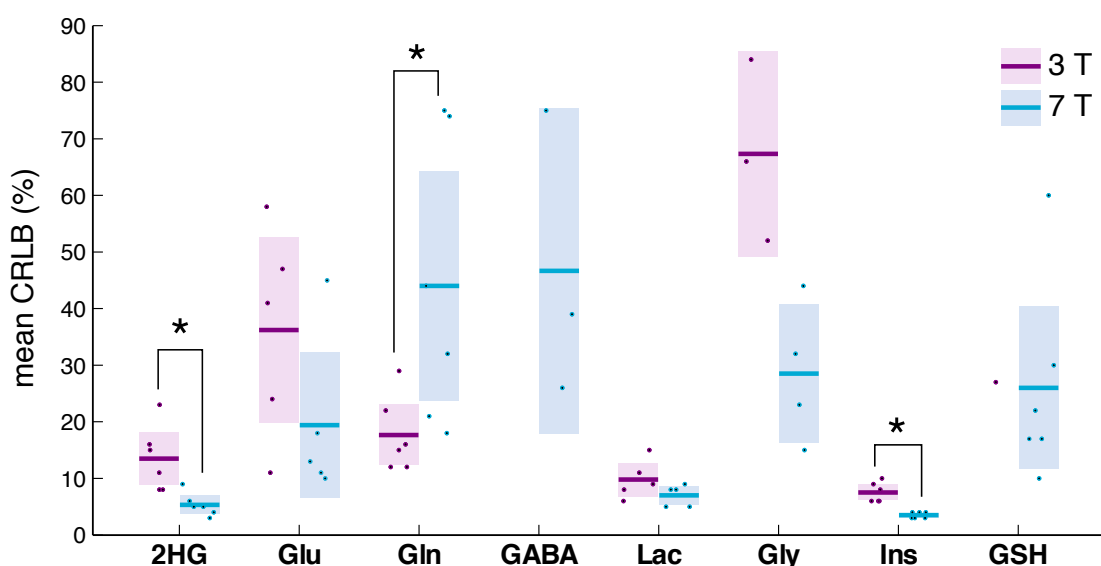


Figure 7.7: CRLBs of key tumour metabolites plotted for each field strength across confirmed IDH-mutated tumour patients. Standard deviation is shown in the highlighted region and statistical differences of $p < 0.05$ are indicated by * using a paired, two-sided t-test.

The relative concentration values in the IDH-mutants, for these detected metabolites, were then compared at 3 T and 7 T. This was performed relative to total creatine

(tCr) and total choline (tCho) in each voxel (see Fig. 7.8(A and B)). The concentrations of each metabolite are in close agreement between field strengths. When referencing to tCr, however, Lac concentration appears significantly different between field strengths ($p = 0.036$), yet this disappears when referencing relative to tCho ($p = 0.12$). In addition, a stronger statistical difference between 2-HG concentrations is observed relative to tCr ($p = 0.066$) compared to tCho ($p = 0.61$). Table 7.1 provides a summary of the relative concentration values for 2-HG fitting and CRLBs for each patient.

At both field strengths, when measured relative to tCr, the largest 2-HG concentration was observed in the IDH1 patient (P07) (14.4 ± 2.3) at 3 T and (17.3 ± 2.8) at 7 T. However, upon visual inspection of the spectra from (P07) in Fig. 7.5, the 2-HG signal is of smaller magnitude than that of P03, while at the same time the creatine singlet is also smaller in this patient. This suggested that relative creatine measures were not accurate estimates of the 2-HG concentration. When referencing to tCho, however, the largest 2-HG concentration is detected in P03 (Table 7.1) at 3 T and 7 T, which matches visual inspection of the spectra. Incidentally, P03 was the only patient in this study found to have an IDH2 mutation. Furthermore, concentrations of Ins were found to be around 40% larger than creatine concentration in the IDH-mutants at both field strengths, similar to work previously reported [365].

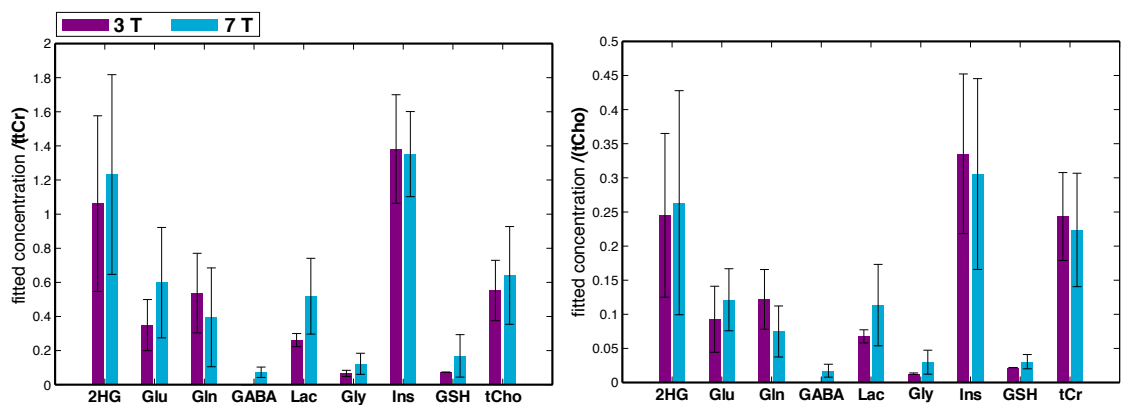


Figure 7.8: Average metabolite concentrations across all IDH-mutants ($n = 6$) relative to total creatine (A) (tCr) and total choline (B) (tCho) for 3 T and 7 T shown with standard deviation. GABA and GSH detected in 0 and 1 patients at 3 T respectively. Statistical significance (*) of $p < 0.05$ is tested using a paired, two-sided t-test

7.5 Statistical Comparisons

To examine the agreement between 2-HG concentrations measured at both field strengths, the correlation and bias between the two methods were analysed using a Bland-Altman analysis on spectra comprising of 64 transients. All IDH-confirmed patients were included in the subsequent analysis along with the IDH unconfirmed patient (P08), who is suspected to be IDH-mutated based on spectral profiles and detected 2-HG concentrations relative to other IDH mutants.

Fig. 7.9A shows the correlation between the ($n = 7$) concentration values of 2-HG measured at 3 T and 7 T, relative to tCr, and reveals a large $R^2 = 0.92$ which is statistically significant ($p = 0.0007$), indicating a high degree of shared variance between 2-HG measured at 3 T and 7 T.

Fig. 7.9B plots the pairwise differences between 3 T and 7 T 2-HG concentrations against their means. The mean difference (bias) was calculated using this method. Confidence intervals calculated from the standard error of the differences reflect 95% uncertainty in the estimation of this bias. When referencing to tCr (7.9B) a significant bias was detected between the methods, with 3 T yielding lower 2-HG/tCr ratios than at 7 T (-1.7 ± 1.6 , mean $\pm$ CI), whereby the upper confidence interval of this mean bias estimate lies marginally below the zero difference line.

The identical analysis was then conducted using the relative tCho values in Fig. 7.9(C and D). A correlation between 2-HG concentrations measured at both field strengths was also detected ($R^2 = 0.68$, $p = 0.02$). However, 2-HG concentrations appeared more clustered than in the tCr analysis, which can also be seen in the Bland-Altman plot of Fig. 7.9D. Interestingly, this clustering was formed by patients with IDH1 mutation status (including unconfirmed patient) and the largest measured concentration was identified as the IDH2 (P03) mutant relative to tCho. The bias was found to be much smaller (-0.03 ± 0.64) than referencing to tCr and was significant with the measured sample size ($n = 7$).

A final exploration of the data acquired with semi-LASER at 3 T and 7 T was performed with principal component analysis (PCA). This focused on measured concentrations of 11 metabolites (2-HG, Ins, Lac, Glu, Gln, GABA, GSH, Gly, tNAA, sIns

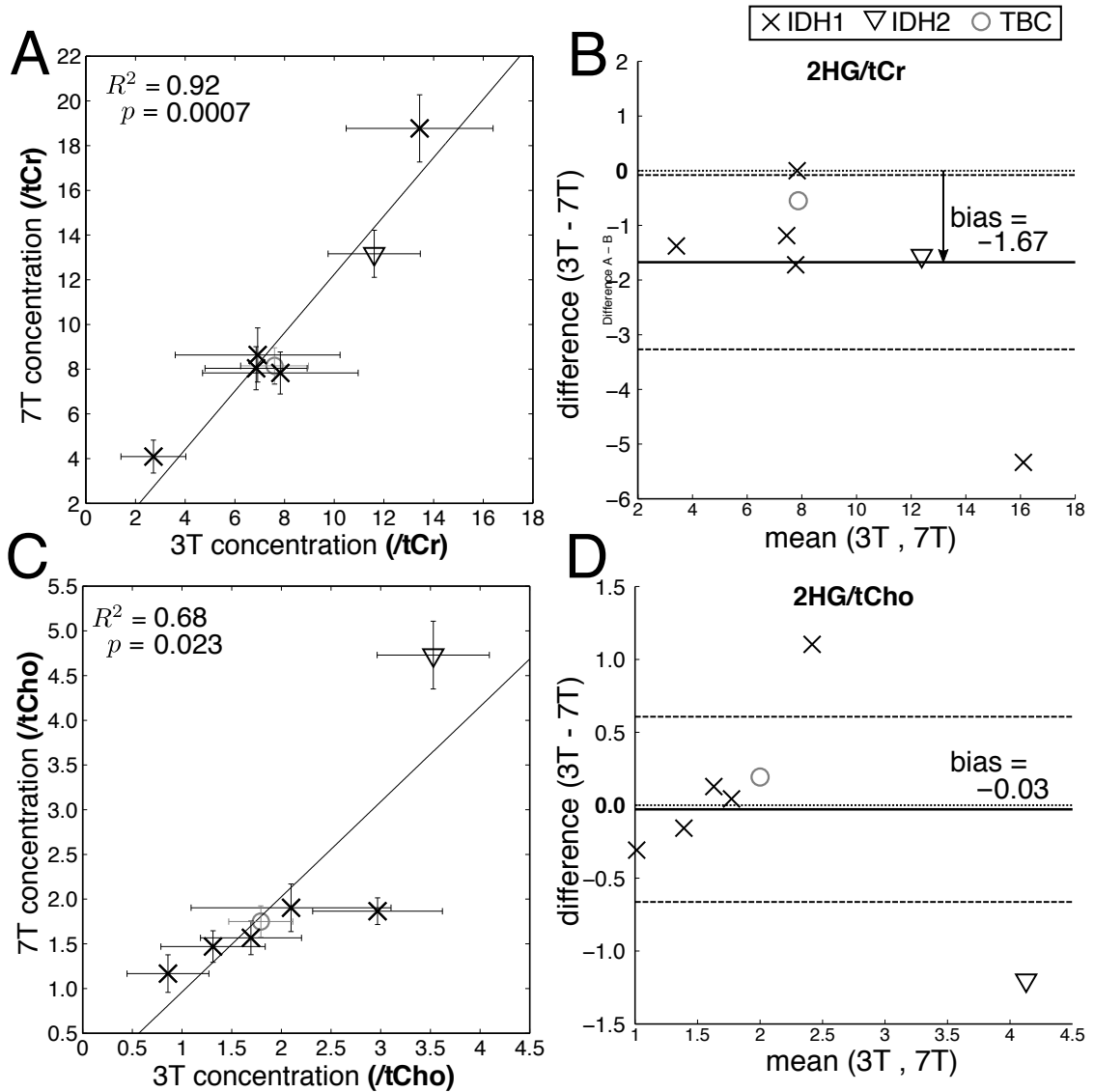


Figure 7.9: Statistical agreement of 2-HG assignment between 3 T and 7 T. **(A and C):** Correlation between the LCModel estimated concentration relative to total creatine (A) and choline (C). Error bars represent 95% confidence intervals. **(B and D):** Bland-Altman analysis of 2-HG concentrations relative to total creatine (B) and choline (D). The means of the measured 2-HG concentrations are plotted against their differences for each patient. The bias (solid line) between the two methods is shown with standard error (dotted line). The patient with unconfirmed IDH-status (TBC, P08) is included in both analyses (circle).

and tCr) for all of the patients in the study ($n = 8$). PCA was performed on this matrix of concentrations relative to tCho (relative tCr measures were also examined but did not produce clear clustering). Concentrations considered undetected ($\text{CRLB} > 100\%$) were set to zero in the PCA and statistical analysis was performed in Matlab (Mathworks, Inc, Natick MA).

Fig. 7.10 shows the results of this at 3 T and 7 T. After calculation of the principal components, each patient was projected onto a space defined by the two first principal components, PC_1 and PC_2 . The loadings of each metabolite on the first two principal components are also shown on the plot as vectors. The proportion of variance captured by each principal component was also calculated and shown for the first four principal components in Fig. 7.10.

At 7 T, the first principal component was found to be $PC_1 = 0.70[2\text{-HG}] + 0.62[\text{Ins}] + 0.25[\text{Lac}]$ for the first three largest loadings. For 3 T, this was $PC_1 = 0.80[\text{tNAA}] - 0.34[\text{Ins}] + 0.25[\text{Glu}]$. Thus, there was no large contribution from 2-HG to the first principal component at 3 T. The total variance captured by PC_1 was 67% at 7 T compared to just 44% at 3 T. This results of the PCA can be seen on the plots in Fig. 7.10. Along the first principal component, clusters of patients corresponding to IDH-status were observed corresponding to IDH1, IDH2 and IDH-WT patients at 7 T. The same clustering was observed when the second principal component is included at 3 T to a weaker extent. Separation into clusters at 7 T was, therefore, driven largely by PC_1 and in particular 2-HG concentration together with Ins. The projection of tCr along PC_2 was large at 7 T and PC_2 was seen to drive separation between wild-type and IDH1/2 mutation explaining 18% of the variance. For 3 T, the second principal component contains a large loading from 2-HG and was seen to separate IDH1 from IDH2 mutations.

Further IDH-mutated patients, and in particular IDH2 patients, would be needed to test the reliability of these metrics for classification. It would be interesting to investigate the possibility of identifying further mutational subtypes with this technique. It is also worth noting that no T_2 correction on relative concentrations has been performed here, which may explain some of the variation between field strengths and also between tumour types.

7.6 Discussion

This work compared MRS acquisition with semi-LASER (110 ms) at 3 T and 7 T for detection of metabolites, specifically the biomarker 2-HG, *in vivo* in tumour patients.

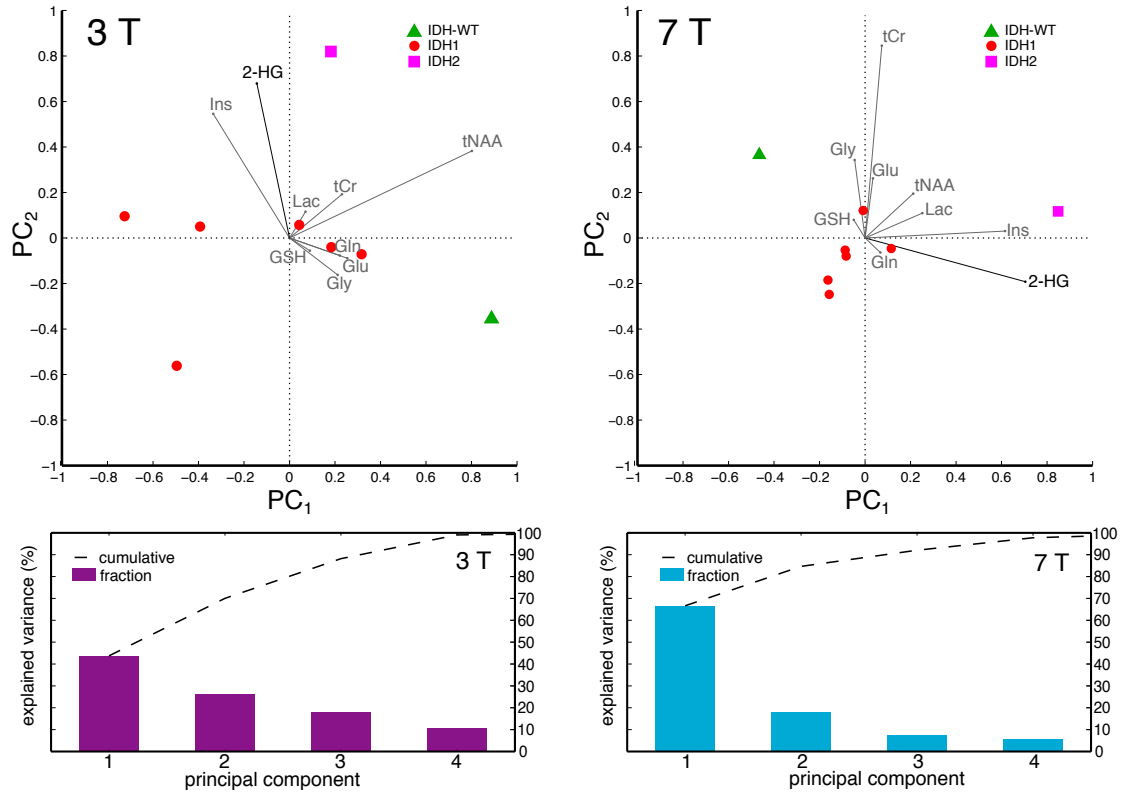


Figure 7.10: Exploratory principal component analysis (PCA) on the observed concentrations from all patients using concentrations of 2-HG, Ins, Lac, Glu, Gln, GSH, Gly, tNAA and tCr. PCA is carried out on 3 T and 7 T data. The first two principal components, PC₁ and PC₂, are plotted and the contributions of each metabolite to these components is displayed as a vectors. After performing the PCA each data point is labelled with corresponding IDH status. Below each plot the cumulative and fractional variance captured by the first four principal components is shown. At 7 T, > 80% of the variance is captured using the first two principal components and clusters reflecting underlying IDH tumour status are found.

Using semi-LASER localisation developed in the previous chapter (Ch. 6), together with a similar sequence optimised at 7 T [365], the data from phantoms and also eight glioma patients, scanned using these sequences, were analysed. Differences between the two acquisitions were minimised by using similar RF coils and data analysis pipelines. The intrinsic signal-to-noise ratio increase at 7 T, together with an increase in spectral resolution, resulted in consistently improved CRLBs of 2-HG fitting in phantom studies, even for low 2-HG concentrations (~ 0.5 mM). This boost in sensitivity at 7 T allowed for significantly lower error in the fitting of 2-HG signal across the IDH-mutants *in vivo* of $(5 \pm 2)\%$, which ultimately led to a need for fewer spectral acquisitions to achieve similar detection than at 3 T. The superior quantification of other metabolites such

as Lac, Ins and GSH at 7 T, also allowed for unsupervised clustering of IDH subtypes based on metabolite concentrations. Thus, the potential exists for 7 T semi-LASER to generate a more reliable and integrated picture of mutation status *in vivo*.

Water Residuals and Field Inhomogeneity in Phantom

Significant water residual artefacts were observed to influence some of the phantom spectra at 7 T (Fig. 7.3C). These artefacts are attributed to stronger localised shim solutions necessary in the phantom at 7 T compared to 3 T. In work by Carlsson *et al.* the origin of the water refocussing artefact is visualised using images of water suppression [314]. Localised shimming in MRS can shift the water resonance away from the suppression bandwidth and lead to a refocussing of water from outside the VOI. Inhomogeneity in the static field (B_0), arising from susceptibility gradients at interfaces are worse at higher field [52] and stronger shim terms are thus required. A two-fold larger spoiling gradient at 7 T, compared to 3 T, would be needed to spoil this spurious signal effectively, although the total echo time and eddy currents may increase as a result. Alternatively, the order of slice excitation may be optimised depending on the voxel position and size as well as the sample [314]. The shape, construction (i.e. presence of air bubbles) and alignment of the phantom in the main magnetic field may, therefore, influence these artefacts. Since the focus of this work was not to optimise the existing 7 T implementation, spurious water resonances were removed post-acquisition by means of the HLSVD method (Fig. 7.3). Poor water suppression can also result from the inhomogeneous B_1 distribution observed in the phantom at 7 T (Fig. 7.3A, dark and light patches). This combination of interference in the receive (B_1^-) and transmit (B_1^+) fields is well described at ultra-high field [56]. In the *in vivo* studies, only one spectrum from 7 T was seen to suffer from poor water suppression and large baseline artefact. This improvement may be due to the use of the BaTiO₃ dielectric pad [59] in the patient studies, thereby increasing B_1^+ transmit efficiency to this region.

Effect of field strength on CRLBs

At 7 T, a significant increase in the sensitivity of semi-LASER to detect 2-HG compared to 3 T was found through analysis of the Cramér-Rao Lower Bounds in phantoms and

in vivo. Assessing the reduction in CRLBs of metabolite fitting has been previously used when comparing field strengths [49, 384]. Lower CLRBS of 2-HG fitting were found consistently across all concentrations in the phantom studies (Fig. 7.4), but also in the case of *in vivo* spectra (Fig. 7.6). Naturally, this is expected since the theoretical SNR increase is linearly proportional to the increase in field strength. For small 2-HG concentrations (0.1, 0.5, 1 mM), CRLBs reached a ‘detection-threshold’, failing to fit small concentrations. Conversely, for large concentrations with high confidence of fitting, CRLBs are rounded by LCMoel and, thus, the precision of CRLBs is not high enough to capture further confidence of fitting with a larger number of spectral averages [384]. This effect can be seen in Fig. 7.4A. For 2mM of 2-HG, the CRLB variation with the number of averages was linear and inversely proportional. Across all spectral averages, mean CRLBs were (1.83 ± 0.09) times lower at 7 T than at 3 T. Cavasilla *et al.* have shown that for singlet resonances the Cramér-Rao Bounds are proportional to the apparent decay rate T_2^* such that $\text{CRLB} \propto T_2^{*3/2}$ and inversely proportional to the SNR, $\text{CRLB} \propto \text{SNR}^{-1}$ [383]. Under the assumption that metabolite line-widths ($\propto 1/T_2^*$) increase linearly with field strength [50], it follows that $\text{CRLB} \propto 1/\sqrt{B_0}$. CRLBs would, thus, be expected to be 1.5 times lower at 7 T than 3 T. Deelchand *et al.*, however, found that for complex coupled spin-systems of Glu and Gln this relationship did not hold and instead found larger CRLB decreases by moving to higher fields, namely $\text{CRLB} \propto 1/B_0$ [384]. This drop in CRLB with field strength equates to 2.3 times lower CRLBs at 7 T.

The observations of this work, in phantoms, fell between these estimates of CRLB improvements. When considering the mean CRLBs of 2-HG *in vivo* (Fig. 7.6B), the reduction at 7 T is greater than in phantom studies. In particular, across all averages the mean CRLB of 2-HG in IDH-mutated patients is 3.6 ± 1.2 times lower than at 3 T. Both increases (in phantom and *in vivo*) are, thus, attributed to more than simply an increase in intrinsic SNR ($\propto B_0$), but also the superior spectral resolution offered at 7 T. This allows far superior separation of the multiplets of 2-HG amongst the crowded resonances, an effect that is noticed predominantly during *in vivo* acquisition, where overlaps are large. A secondary reason for the large boost in CRLB could be similar

to that stated by Tkáč *et al.*, who reported an increase in SNR greater than theoretical expectations at 7 T compared to 4 T, which they suggest may be a result of poorer B_1^- penetration at 7 T and, therefore, a smaller region contributing to noise in the receiver [49, 387].

CRLBs at 7 T were significantly improved for the coupled spin-system of 2-HG *in vivo*. This was observed in the spectra of patient P03 (Fig. 7.6A), where the inverted peak of 2-HG at 7 T was visible even after 2 averages, corresponding to a 12 s acquisition *in vivo*. Over the IDH-mutants this was equivalent to a 62% reduction in scan time compared to 3 T to achieve CRLBs < 20%. Acquisition at 7 T may, therefore, be advantageous over 3 T for use in busy clinical cancer imaging protocols.

This sensitivity increase also results in a clearer separation of 2-HG assignment in the IDH-WT patient from the IDH-mutated patients *in vivo*, with CRLBs for WT of 40% and 64% at 3 T and 7 T, respectively. In the confirmed IDH-mutant tumours, 2-HG was shown to be quantified with significantly lower CRLBs at 7 T (5 ± 2)% compared to 3 T (14 ± 5)% ($p = 0.009$).

Other Metabolites

This work also focused on how the detection of other neurochemical markers, which may be important in the diagnosis of tumours, is improved in IDH-mutated gliomas at 7 T. Firstly, significantly reduced *myo*-inositol (Ins) CRLBs were found at 7 T (2.3 ± 0.5)% compared to 3 T (4.2 ± 1.3)% ($p = 0.002$). The measurement of Ins is made challenging because of spectral overlaps since Ins has a triplet resonance at 4.05 ppm, close to the H2 resonance of 2-HG, and its two strongest resonances lie at 3.61 ppm and 3.52 ppm, which overlap with the glycine (Gly) singlet at 3.55 ppm [313]. Increased levels of Gly have also been implicated in recurrent gliomas [388] and as such may reflect tumour malignancy. However, it remains uncertain whether assigned Ins concentrations in this work contains large contributions from Gly, even at 7 T. Optimised acquisition strategies at 3 T [389] and also 7 T [390, 391] have been proposed to separate Gly from Ins achieving CRLBs of $\sim 7\%$. CRLBs for Gly in this study were, thus, considerably larger

reflecting the uncertainty at 3 T ($67 \pm 16\%$) and 7 T ($29 \pm 12\%$) across IDH-mutants because of these overlaps, with Gly detected in one additional patient at 7 T.

In addition, glutathione (GSH) was detected in all IDH-mutated patients at 7 T, but only one patient at 3 T. The sensitivity at 3 T to detect this metabolite is reduced partly because of significant overlaps with Glu and Gln [313]. It has been recently shown at 9.4 T, that GSH concentration may be lower in IDH-mutants compared to wildtype [354]. This drop in GSH, through a decrease in NADPH availability in the cell, increases oxidative stress and, hence, susceptibility of the tumour to chemotherapeutic agents [379]. GSH is, therefore, an important avenue for future studies of 2-HG *in vivo* and, thus, 7 T offers the potential for monitoring the levels of this cellular antioxidant in IDH-mutants.

The analysis also revealed significantly *larger* CRLBs of Gln fitting at 7 T than 3 T. This observation may be driven by the fact that separation of Gln from Glu at 3 T is not possible using single shot techniques, thus the Gln signal is susceptible to overfitting at 3 T [54, 392]. Using spectral simulations, Deelchand *et al.* found that individual measurements of Gln and Glu were not estimated reliably at 3 T [384]. Whereas at 7 T, the separation of the H4 spins of Gln and Glu is sufficient to resolve signals [29]. Therefore, the LCModel measure of Gln at 3 T may contain significant Glu contribution. Mean concentrations of Gln were also found to be higher at 3 T than 7 T, whereas Glu concentrations were lower, although neither reached significance (Fig. 7.8). Previous studies of IDH-mutated tumours have found decreases in Glu relative to wild-type [354, 365] and also decreases in Gln in cell models [284]. The larger fitting error at 7 T may also accurately reflect the small concentrations of Gln in IDH-mutated tumours [284].

As a result of the optimisation of the semi-LASER sequence at 3 T, presented in Chapter 6, a large overlap with GABA resonance at 1.89 ppm was discussed which generates problems for accurate fitting of 2-HG. It was, therefore, possible that the increased spectral resolution offered at 7 T could help distinguish between these resonances. In this study, GABA was fit in three patients at 7 T, whereas it failed to be fit in any patient at 3 T. The failure to fit GABA may suggest that the 3 T measurements of 2-HG contain signal contribution which is stolen from the GABA resonance. However,

spectral overlap is still problematic even at 7 T. Ganji *et al.* removed 2-HG from the fitted basis at 7 T and found significant differences in the assigned GABA concentration. Thus, although GABA is assigned at 7 T in some tumour patients, it is not clear to what extent this measure reflects true concentration. It is, therefore, proposed that extensive work using phantom studies, simulation and spectral editing sequences *in vivo* is carried out, in order to fully establish the effect of GABA on 2-HG at 3 T and 7 T.

Longitudinal Relaxation, T_1

One limitation of the current comparison is the use of different repetition times at 3 T (TR = 3 s) and 7 T (TR = 5.9 s). Since *in vivo* comparisons were made during a busy clinical protocol, the TR was shorter at 3 T in order to reduce the total scan time. However, in order to minimise influence of T_1 -relaxation, TRs should be optimised at each field strength. T_1 -relaxation rates of metabolites are known to increase with static field strength B_0 [304]. For example T_1 values for NAA in healthy brain at 3 T and 7 T are 1380 ms [393] and 1730 ms [371], respectively. T_1 values in gliomas were also found to be similar to healthy brain tissue [393]. The amount of signal attenuation due to T_1 -relaxation at 3 T compared to the signal observed at 7 T can be calculated for NAA as

$$1 - \frac{(1 - e^{TR/T_1(NAA)})_{3T}}{(1 - e^{TR/T_1(NAA)})_{7T}} \times 100\% = 8.7\%. \quad (7.1)$$

This difference in signal attenuation (8.7% for NAA) would result in a greater underestimation of metabolite concentration at 3 T if absolute concentrations were reported (neglecting differences in T_2 decay). Values for the T_1 of 2-HG at either field strength are not currently known making TR optimisation difficult. Concentrations in this study were, therefore, reported relative to creatine or choline signals and it is expected that T_2 -decay, rather than T_1 , will drive the majority of the signal variation between field strengths.

Transverse Decay, T_2

For the analysis of concentrations, no correction for T_2 -decay of metabolites was applied in this work. However, at the long echo time used in this study (TE = 110 ms), T_2 -weighting of spectra is considerable. T_2 -decay becomes greater with increasing

field strength [50, 304] and T_2 -decay rates also vary between metabolites by 20-30% [394]. In addition, factors such as the tissue content in different brain regions [395], and across tumours [363, 369], significantly influence T_2 -decay values. Apparent T_2 values also change depending on the sequence used [73] and values for hard-to-measure, overlapping, J-coupled metabolites are rare or absent from the literature [396]. Rather than introducing further variability in the concentrations by using estimated T_2 s, concentrations were referenced relative to either total creatine (tCr) or total choline (tCho) in each voxel. Internal referencing is valid under the following assumptions:

1. T_2 values are similar between the reference peak (Cr or Cho) and the metabolite of interest,
2. T_2 values of the reference peak (Cr or Cho) and the metabolite of interest, m decrease in the same relative proportion moving from 3 T to 7 T, namely

$$\frac{T_2(m)|_{7T}}{T_2(m)|_{3T}} = \frac{T_2(\text{ref})|_{7T}}{T_2(\text{ref})|_{3T}} = \text{const.} \quad (7.2)$$

Since the first assumption does not hold, individual metabolite differences within a tumour reported in this work may be driven by T_2 differences between the reference and metabolite of interest. Therefore, the interpretations of concentrations in this work is limited.

The purpose of this study was, however, to compare detection using long TE semi-LASER over 3 T and 7 T and not the accuracy of concentration estimation. Li *et al.* have measured T_2 differences between 3 T and 7 T in healthy brain and report values for Cr of 150 ms and 120 ms, and NAA of 262 ms and 170 ms at 3 T and 7 T, respectively (using a PRESS sequence) [371]. These are equivalent to relative T_2 decreases at 7 T of 24% and 35% for Cr and NAA, respectively. In terms of signal decay, at TE = 110ms, it is calculated that NAA will have decayed by 36% less than creatine signal at 3 T and this value is 30% at 7 T. This decay results in a difference across field strengths of 6%. The observation that 2-HG/tCr underestimated at 3 T compared to 7 T (or equally for 7 T to overestimate) may be due to this differential T_2 -relaxation (see Fig. 7.9). In particular,

there was almost no difference in 2-HG measured at 3 T and 7 T when referencing to total choline (tCho, -0.03 ± 0.64), instead of total creatine (tCr, -1.7 ± 1.6).

Differential T_2 decay for metabolites and reference between 3 T and 7 T may also explain the observation of a significant Lac/tCr concentration difference (Fig. 7.8A), but not between Lac/tCho measures (Fig. 7.8B). These findings may indicate that the relative difference in T_2 between 2-HG and Cho is more similar compared to 2-HG and Cr.

Relative choline measures also led to a better separation of IDH1, IDH2 and wild-type patients (Fig. 7.10 and Table 7.1), since the patient with the highest 2-HG/tCho concentration corresponded to the largest observable 2-HG resonance in the spectra. This tumour was incidentally also found to be IDH2 mutated.

For the semi-LASER sequence in this work, T_2 signal decay is, however, reduced due to multiple refocussing. T_2 -values have been shown to be twice as large using a CP-LASER scheme compared to using PRESS localisation [73]. Therefore, T_2 -weighting may be significantly lower than discussed here, even at TE = 110 ms. For complex J-coupled resonances, such as 2-HG, T_2 s are difficult to measure and certainly not feasible *in vivo* during a clinical protocol. However, the use of a TE-averaged acquisition prior to MRS may be an avenue for future research using the techniques to measure individual relaxation of creatine or choline singlets *in vivo* for accurate concentration estimates.

7 T and *in vivo* metabolomics

By performing a PCA on the metabolite concentrations relative to tCho, a clustering of patients based on IDH status was observed. Separation of IDH1, IDH2 and IDH-WT patients was observed, particularly at 7 T, and may be driven by the T_2 differences across these tumours (discussed above). The very low number of patients studied in this work, in particular IDH-WT ($n = 1$) and IDH2-mutated ($n = 1$) patients, means that statements about separation and clustering based on IDH-status are very far from being statistically conclusive. Nevertheless, analysis of the loadings of each principal component reflects concentration changes previously reported in the literature [354].

At 7 T, Cr, measured relative to Cho (tCr/tCho), had a large loading on the second principal component (Fig. 7.10). Separation of IDH-wildtype and IDH1/2 mutants was possible using this component alone. The clustering along this axis reflects a decrease in Cr/Cho for IDH-mutants. Interestingly, the inverse of this ratio (Cho/Cr) is already used widely in the literature for tumour staging and discrimination between tumour types [79, 397–399]. Elevated Cho reflects increased proliferation and turnover of phospholipids in tumours undergoing malignant transformation [400, 401]. It has also been shown that Cho/Cr is elevated in IDH-mutants relative to wild-type at 9.4 T [354], similar to the observations discussed here.

Rather than being driven by Cho, however, differences between IDH-mutants and wild-type may also be driven by changes in Cr, and in particular a drop in Cr/Cho ratio. Heiland *et al.* have recently performed work integrating ^1H -MRS concentrations with gene expression data in GBMs [402]. They find clusters of genes associated with different Cr concentrations (normalised to concentrations in normal white matter). In particular one of the strongest links appears between a genetic subtype (pro-neural) marked by IDH1 mutation and very low Cr levels. Low levels of Cr were attributed to severe disruption of the TCA cycle, and hence drop in intra-cellular creatine in IDH1-mutants (no data were obtained from the rarer IDH2 mutation). Since referencing to creatine is standard in MRS of tumours, differences in the level of this metabolite have the potential to be overlooked. In particular, it is hypothesised that for the sub-classification of IDH-subtypes, such information may be needed to reflect the spread of impaired metabolism across different mutations.

In terms of its comparison to 3 T, the PCA at 7 T generated a clearer separation between IDH-status using one principal component expressing (67%) of the variance. This is attributed to improved detection of the underlying 2-HG concentration, which has the greatest loading of this component, compared to 3 T. In addition, the majority of the variance at 3 T (first principal component) is accounted for with a large NAA loading rather than 2-HG, which suggested a separation between IDH-WT from IDH-mutant tumours. Finally, in the PCA analysis performed in this work, reported concentrations may be affected by different receive intensities in each tumour voxel,

thus l_2 normalisation could be performed on the spectra in future work, prior to PCA analysis, to remove such signal differences [403].

Long-TE MRS at 7 T may be better suited to perform metabolomic analysis for the classification of IDH-mutation. It is expected that the more accurate reflection of concentration values at 7 T, reflected by reduced CRLBs, will be able to tease apart metabolic differences between IDH-WT and IDH-mutant tumours. The exploratory analysis is, however, severely limited in this work by the small sample size (IDH2: $n = 1$, IDH-WT: $n = 1$). Further analysis on a larger cohort as well as the inclusion of more metabolic concentrations and mutational information may reveal subtle links between tumour-type and metabolism *in vivo*.

7.7 Conclusion

This chapter has shown that 7 T semi-LASER is able to robustly detect 2-HG in IDH-mutants compared to 3 T. A larger than expected increase in sensitivity for detection, owing to the better resolution at 7 T and greater separation of complex multiplets, greatly benefits 2-HG, but also other metabolites such as GSH, Lac, Gly. Performing an integrated analysis over these detected concentrations led to a distinct separation of patients based on mutational status when referencing to total choline. Further studies on measuring relaxation properties of metabolites at 7 T and increasing the sample size of patients is needed to fully explain differences reported here. 7 T may offer the ability for improved *in vivo* metabolomic analysis of IDH-mutants and other tumour mutations compared to acquisition at 3 T.

8.1 Summary

The work presented in this thesis was motivated by the consequences of metabolic alterations in the living brain. The aim was to develop methods for ^1H -MRS capable of sensitive neurochemical measurements *in vivo*, largely by minimising the impacts of poor localisation on the spectral acquisition. Firstly, through modification of a semi-LASER MRS localisation scheme, a two-voxel method was proposed and implemented in a novel neurochemical study of positive and negative BOLD responses. Secondly, optimisation of semi-LASER localisation was carried out for the detection of the oncometabolite 2-HG in glioma patients. Detection of 2-HG was also compared between 3 T and 7 T, demonstrating significant advantages for metabolic measurement of glioma. It is hoped that this work can contribute to both clinical diagnosis and understanding as well as basic neuroscience research. Furthermore, the methods presented in this work may hopefully spark further development on improving localisation methods for MRS, in particular over multiple spatial locations, for detection of metabolic alterations in the healthy and diseased brain.

Hadamard encoding is an established technique for simultaneous encoding of multiple locations within a sample. In this thesis, a two-voxel Hadamard encoded excitation was proposed within an adiabatic localisation scheme. Modification of short duration asymmetric RF excitation pulses resulted in excitation profiles as determined by simulation and then *in vivo*. The amount of signal bleed between voxels was lower than previously described in the literature using conventional sinc pulses [97]. Importantly, the technique presented here suffered from low chemical shift displacement (1.35 mm/ppm in excitation direction, bandwidth 2.2 kHz per voxel) and together with the use of broadband adiabatic inversion pulses (0.56 mm/ppm

refocusing direction, bandwidth = 5.3 kHz), the total chemical shift in three dimensions was minimised. Applying the technique *in vivo* resulted in high quality comparable *in vivo* spectra from both voxels. In order to extend the technique presented in this chapter to wider applications at 7 T, greater spatial coverage may be desired. The use of SENSE based encoding of MRS voxels [318] together with RF pulse design based on VERSE [321] may lead to the ability to perform multiple encodings with sufficient bandwidth while at the same time reducing overall RF power constraints. It is hoped that this would lead to the development of general multi-banded MRS approaches in the future. Such techniques would allow for efficient sampling of spatial and temporal metabolic changes across the brain.

The Hadamard method was implemented in a two-voxel functional-MRS study in this thesis. However, it is anticipated that the method offers the potential for robust two-voxel MRS measurement at 7 T for other purposes. For example, the technique may benefit measurement in tumour when comparison to a control region of healthy tissue is required [78]. It is also envisaged that neuroscience studies that desire measurement of neurochemical changes from different brain regions may benefit from such a technique.

After demonstrating the technique in healthy volunteers the two-voxel method was used in the study of the BOLD response. The aim of this study was to determine neurometabolic changes associated with sustained negative BOLD responses (NBRs) and compare them to the positive response in humans. A survey of the literature revealed that NBRs are likely to reflect regions of suppression and neuronal deactivation, partly evidenced by concomitant decreases in neural activity in such regions [205]. A hypothesis was therefore constructed, which suggested that concentrations of inhibitory neurotransmitter GABA may transiently increase in NBR regions arising from increases in local inhibition. The absence of any significant changes in GABA, in the results of Chapter 5, however, did not support this hypothesis. Rather, the results serve to suggest that negative BOLD in visual cortex may not be driven by large observable changes in GABA concentration. In addition, concomitant Gln increases upon activation were found in NBR timecourses, which were negatively correlated to

that of the PBR. An unexpected decrease in ascorbate concentration was observed after performing a correction for the BOLD-induced linewidth changes. Ascorbate has been linked to glutamatergic function in the brain via inhibition of glucose transporters to neurons [342]. Astrocytes have also previously been shown to release ascorbate upon glutamate stimulation [341]. A decrease in ascorbate during functional activation may, thus, signal reduced glutamatergic activity and thus neuronal activity. However, the mechanism behind this ascorbate decrease raises new questions for the NBR and care must be taken to ensure significance is not a result of the linewidth correction applied in this work. The results of this study indicate that neuronal suppression in NBR may reflect decreased glutamatergic activity, and by extension, an ascorbate drop. However, given the coupling between excitatory and inhibitory synaptic processes and the sensitivity of proton MRS to detect metabolic changes in these systems, changes in GABAergic inhibition in NBR regions cannot be ruled out. Neurochemical changes and timecourses of increased Lac, Glu and decreased Glc+Tau (a surrogate marker of glucose) were in agreement with previous changes mentioned in the literature [177, 180, 236] and indicated an increased flux through oxidative metabolic pathways as has been previously described [128]. As well as these metabolic changes, the results of this study demonstrated T_2^* line-broadening in half of the NBR voxels (0.12 ± 0.24) Hz, which was confirmed to be statistically different than the line-narrowing observed in the positive BOLD response. The rejection of several spectra due to contamination in this study weakened the available statistical power and this was exacerbated by smaller voxel size in this two-voxel study compared to previous fMRS studies [180]. These results demonstrated the first measurements of *in vivo* neurochemical changes during NBRs in humans. Future work may endeavour to reduce the contamination of individual BOLD responses and macromolecular contamination, through use of dynamically shimmed voxels and improved positioning. In particular, an increase in statistical power is also needed since the BOLD amplitude of NBRs is smaller than PBR. Future work may endeavour to combine fMRI acquisition with MRS in NBR regions, as has recently been shown [331], thereby obtaining simultaneous BOLD maps together with neurochemical changes.

Adiabatic localisation was then also implemented for the measurement of metabolic alterations in glioma. The total echo time of a semi-LASER scheme was optimised for the detection of 2-HG in IDH-mutated tumours, with the aim of improving refocussing and assignment of the 1.9 ppm peak of 2-HG amongst crowded resonances of Glu and Gln at 3 T. Density matrix simulations of the strongly coupled spin system explicitly revealed that the use of a similar echo-time PRESS localisation ($TE = 97$ ms) fails to fully refocus the 1.9 ppm resonance [345, 356] owing to the generation of large compartmentalisation of the signal within the voxel from the chemical shift displacement artefact. Adiabatic refocussing with semi-LASER at an optimised TE of 110 ms generated far superior localisation of the signal (refocusing bandwidth = 5.5 kHz) with reduced overlap with Glu and Gln as measured by pair-wise fitting correlations. Application of the technique in phantoms and, later, glioma patients revealed that the confidence intervals in 2-HG fitting was reduced using semi-LASER, however issues with overlap from overlapping GABA resonances still remained. Nevertheless the amount of GABA within tumours remains unclear, thus further work on measuring GABA content in tumours is needed. This work provided a clear demonstration of the importance of considering the effect of poor localisation on the spectral profile of coupled spin systems and the benefit that may be derived from using adiabatic localisation. It also offers a further tool to researchers and clinicians who wish to measure levels of 2-HG in IDH-mutated tumours at 3 T.

The use of ultra-high field acquisition was then compared to the method presented at 3 T. A handful of sequences were recently proposed at ultra-high field which suggested high sensitivity of 2-HG measurement in glioma patients [354, 365, 374]. Therefore, there was a need to conduct a rigorous analysis of the benefit of 7 T compared to 3 T, with the aim of determining if ultra-high field acquisition can provide a clinically useful benefit compared to 3 T. Phantom studies and data from *in vivo* patients revealed that the enhanced sensitivity at 7 T resulted in much lower fitting errors (3.6-fold). This effect was noticed strongly in the *in vivo* spectra when compared to phantom data, with a greater reduction in fitting error than predicted from a theoretical SNR increase [383]. The large reduction in CRLBs for 2-HG fitting

was, thus, attributed to the effect of increased spectral resolution benefiting the crowded overlapping peaks of 2-HG. At 7 T, separation of IDH-mutants and wildtype was improved and the superior detection of metabolites Lac, Ins and GSH allowed improved fitting of these metabolites. It was also found that referencing concentration values to total choline content yielded results in greater agreement between field strengths and there is potential that separation of individual patients can be achieved according to the mutational status of the tumour. Such a technique would yield insight into metabolic reprogramming in IDH-mutations. However, issues such as the increasing T_2 at 7 T, differences between metabolites and the low number of patients in the study need to be addressed if such conclusions are to be drawn. Further work may, therefore, endeavour to measure T_2 -values of 2-HG and associated metabolites in different tumour environments, thus providing a more accurate quantification. This work has provided a demonstration of the possible clinical benefit of 7 T, and also for performing *in vivo* metabolomics of tumour mutational status with non-invasive measurement.

8.2 Future Work

Common to each of the applications presented in this thesis, an increase in the spatial coverage of MRS measurement would have enabled acquisition of a greater degree of information. For instance, the measurement of metabolite distributions, in particular 2-HG, across whole tumours rather than from a single voxel would potentially allow for assessment of metabolic status across the tumour. For the study of functional changes in the fMRI-BOLD response, an increased spatial coverage would enable spectral acquisition from multiple brain regions during a functional MRS paradigm and would also remove the constraints of acquiring from a single voxel, which can lead to contamination and rejection of data if poorly positioned. MRSI methods endeavour to fulfil this role, however the challenge for such techniques is to maintain the quality of single voxel acquisition. Future work may, therefore, be needed in the development of multi-band spectroscopy similar to simultaneous multislice imaging (SMS) in MRI. In particular, this would be of particular use at 7 T, where the advantages

discussed in this thesis, such as improved fitting for coupled resonances, spectral resolution and SNR, offer a significant benefit for MRS. As previously mentioned, the use of SENSE reconstruction with multiband pulses may allow for encoding of multiple spatial positions in the brain while maintaining high spectral quality [318], however challenges remain in terms of maximum RF power and specific absorption rate (SAR). It is suggested that the recent introduction of parallel transmit technology for MRI may enable the development of multiband pulses for such MRS studies, which constrain the maximum RF power while maintaining large bandwidths to reduce chemical shift displacement [58, 404].

The effect of reducing chemical shift displacement has been the motivation for the use of modified localisation methods with adiabatic refocussing in this work. The use of (frequency offset corrected inversion) FOCI [366] and (gradient-modulated offset-independent adiabaticity) GOIA [60] pulses, however, offers superior chemical shift displacement compared to conventional adiabatic pulses, with the added benefit of a reduction in RF peak power. Future work may, therefore, also focus on adapting these established pulse techniques for use in multi-banded acquisition.

This thesis has provided a demonstration of the importance of robust localisation in MRS for measurement of metabolism in the brain. Through its effect on the coupled spin systems of 2-HG, to the multi-banded approach developed at 7 T, the reduction of chemical shift displacement is fundamental to obtaining accurate metabolic information in the *in vivo* brain. As well as proposing new methods that are robust to poor localisation, this thesis has additionally contributed to the biological understanding of the metabolic state of the healthy and diseased brain through the studies of BOLD responses and metabolic alterations in glioma.

Appendices

The long TE PRESS sequence

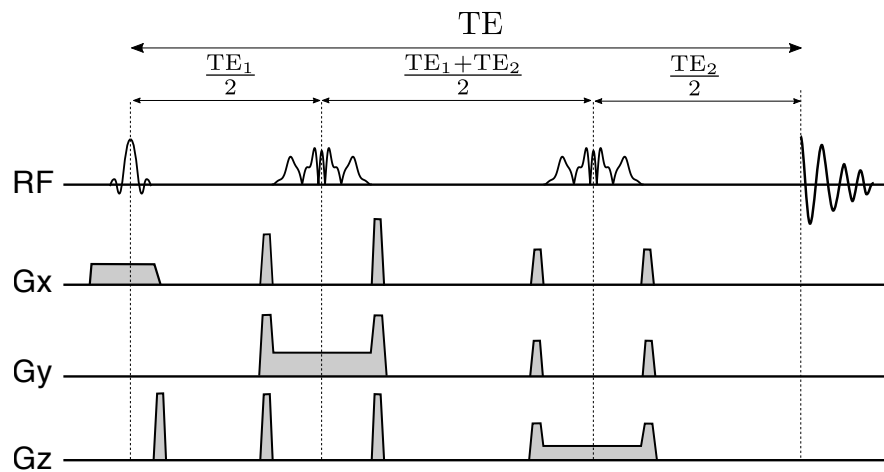


Figure A.1: Asymmetric PRESS localisation scheme with characteristic sub-echo times $TE_1 = 32$ ms, $TE_2 = 65$ ms and total echo time $TE = 97$ ms as proposed by Choi et al. [345].

Fig. A.1 shows the PRESS method used for comparison in Chapter 6, with Refoman6 refocussing pulses. Total echo time TE is 97 ms and two asymmetric sub-echoes are $TE_1 = 32$ ms and $TE_2 = 65$ ms. Crusher gradients were also applied around refocussing pulses. A standard hsinc 90° excitation pulse (duration = 2.56 ms, bandwidth = 3.4 kHz) is used in this work, although Choi et al. originally made use of an amplitude/frequency modulated excitation pulse.

B

HSn adiabatic pulses

Figure B.1 shows the HS4 inversion pulse, with amplitude and frequency modulations together with the resultant longitudinal magnetisation of an on-resonance spin.

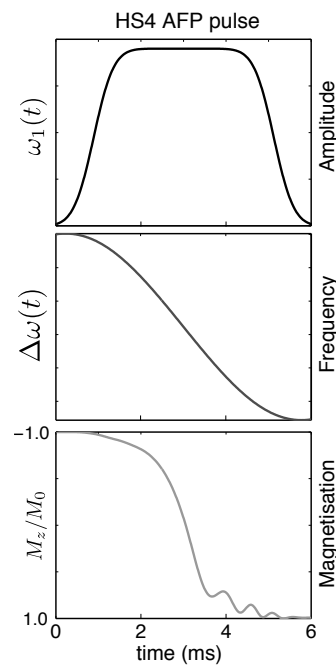


Figure B.1: The hyperbolic-secant 4 adiabatic refocussing pulse (HS4), with amplitude and frequency modulation [65]. **Bottom:** The longitudinal component of the magnetisation for a spin resonating with ω_0 upon application of the pulse.

C

Mean BOLD activations

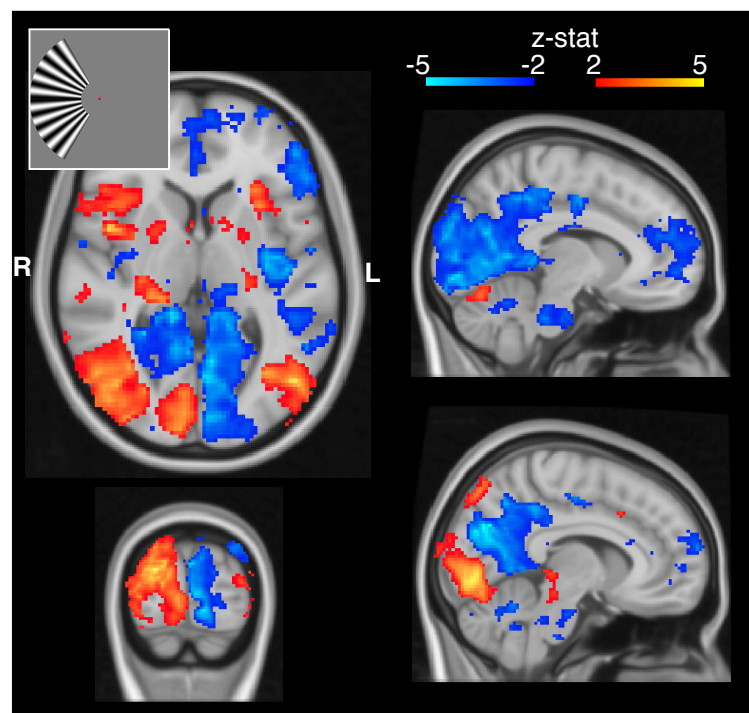


Figure C.1: Mean BOLD activation maps for the left-sided visual stimulus.

D

Analysis of difference spectra

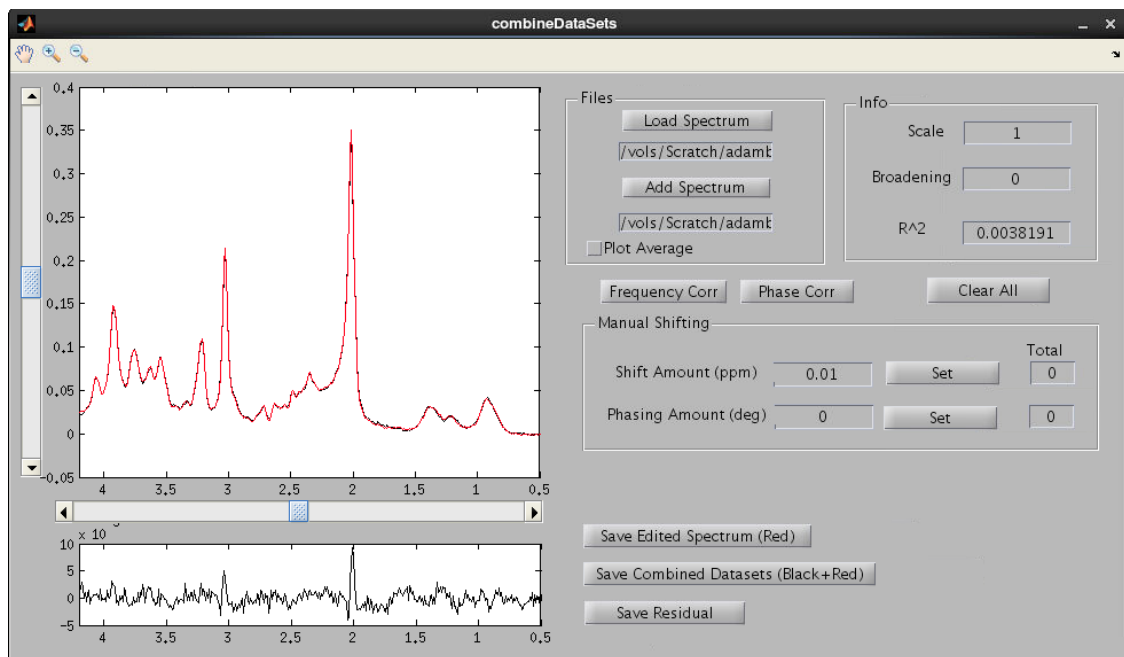


Figure D.1: The software developed for analysis of difference spectra during the functional paradigm. Code written in Matlab (Mathworks, Inc., Natick, MA)

E

Flip-angle profile simulations

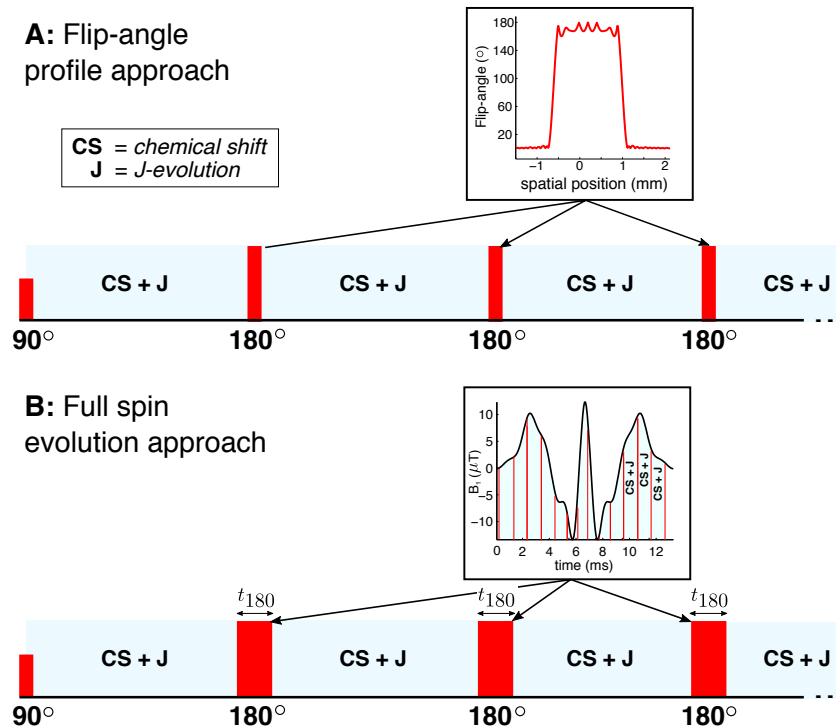


Figure E.1: The difference between the flip-angle profile model (A) and the complete simulations (B). CS = Chemical shift evolution. J = J-evolution.

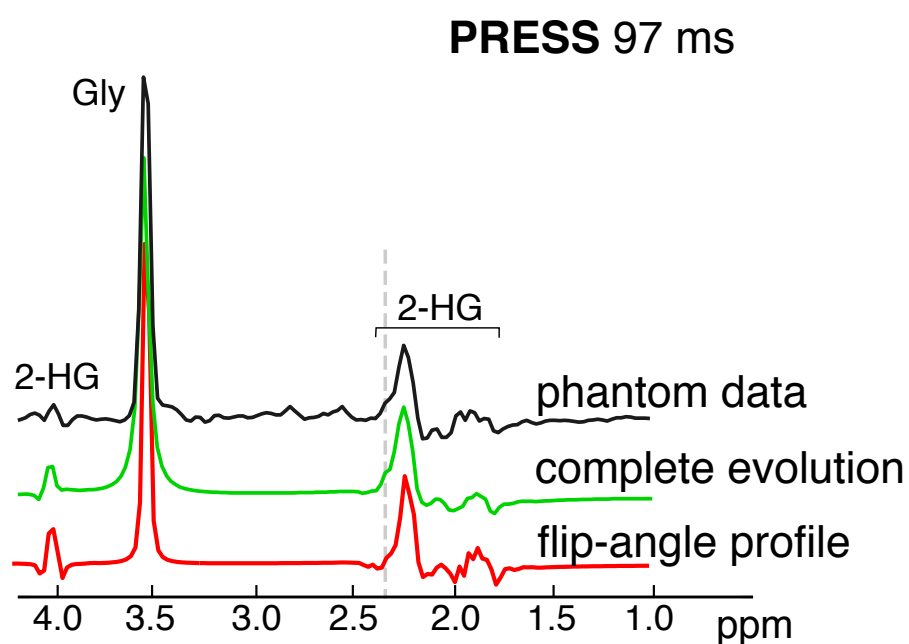


Figure E.2: The failure of the flip angle model compared to complete spin evolution for simulation of 2-HG. Subtle differences in the spectral pattern of 2-HG exist between the simulation methods (grey line), including the lineshape of the peak at 2.25 ppm, which are not fully captured by the flip-angle model. Note, there is a slight difference in linewidth between the spectra displayed here.

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