GABA quantification:

The reliability of GABA measurements in the human brain using MRS at 3T

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Abstract

GABA (γ-aminobutyric acid) is an amino acid neurotransmitter which plays an important role in the central nervous system (CNS). Altered levels of GABA have been linked with epilepsy as well as a number of other neurological disorders. Proton Magnetic Resonance Spectroscopy is a tool that enables measurement of metabolic concentrations in vivo. One of the most commonly employed pulse sequences is the Point-RESolved Spectroscopy sequence, PRESS. However, the spectral signal of GABA is obstructed since its resonance frequencies overlap with strong signals from other metabolites. PRESS does not provide sufficiently high resolution at magnetic field strengths that are normally used clinically (up to 3T). This is why a J-difference editing technique called MEGA-PRESS was employed instead. This sequence was applied on a phantom containing 3.9 mM of GABA (double the concentration in a healthy adult brain) and later on a phantom containing 7.8 mM. In order to obtain the edited GABA spectra, a MATLAB script was written in order to process the raw data from the MR scanner (output in the Siemens DICOM format). The editing of the spectra as well as a number of essential post-processing steps were developed and incorporated into the code. In the phantom study the MEGA-PRESS sequence was able to detect the twofold increase in concentration. Although in both the 3.9 mM phantom and the 7.8 mM phantom the signal only amounted to 50% of the available concentration. The PRESS sequence applied to the 7.8 mM phantom could not reliably detect GABA at all. For in-vivo experiments 8 healthy volunteers (2 female, 6 male) age 24-40 were studied. An attempt was made at quantifying GABA relative the n-acetyl-aspartate (NAA) signal. The biggest challenge posed by studying GABA in-vivo is to obtain a homogeneous magnetic field over the volume of interest (VOI), something that greatly improves the quality of the spectra. Another aspect of GABA studies is obtaining optimal scanner parameters, such as the echo time TE, the repetition time TR, the size and the location of the voxel etc., all of which are addressed in this work.
1 Introduction
1.1 Project overview and objectives .................................. 1
1.2 The basics of MRS .................................................... 2
   1.2.1 Bulk magnetization and relaxation ......................... 3
   1.2.2 Chemical shift .................................................. 4
   1.2.3 J-coupling ...................................................... 5
1.3 Single voxel spectroscopy .......................................... 7
   1.3.1 PRESS ............................................................ 7
   1.3.2 Shimming ....................................................... 8
   1.3.3 Water suppression .............................................. 8
1.4 GABA ................................................................. 9
   1.4.1 GABA-mediated inhibition .................................... 9
   1.4.2 Spectral editing of GABA using MEGA-PRESS .......... 9

2 Experimental .......................................................... 13
2.1 Signal acquisition with MEGA-PRESS ............................. 13
   2.1.1 The FID signal ................................................. 13
   2.1.2 Processing in MATLAB® ...................................... 14
2.2 Phantom study ....................................................... 16
   2.2.1 Attempt at signal quantification using LCModel ........ 17
   2.2.2 Signal quantification using MEGA-PRESS ................. 18
2.3 In-vivo study ........................................................ 24
   2.3.1 Voxel placement ................................................ 24
   2.3.2 In-vivo signal quantification ................................. 27

3 Discussion and Conclusion ........................................... 29
3.1 Choosing the appropriate RF pulse ............................... 29
   3.1.1 Sinc vs Gaussian .............................................. 29
3.2 Effects of magnetic field inhomogeneity ....................... 31
   3.2.1 Magnetic susceptibility ...................................... 31
   3.2.2 The importance of shimming ................................. 31
3.3 The challenges of signal quantification . . . . . . . . . . . . . . 34
3.4 Future applications . . . . . . . . . . . . . . . . . . . . . . . . 34

List of abbreviations 37
Acknowledgments 39
Bibliography 41
Chapter 1

Introduction

1.1 Project overview and objectives

Proton Magnetic Resonance Spectroscopy ($^1$H MRS) is a non-invasive technique that allows detection of different metabolite concentrations inside the human body. GABA ($\gamma$-aminobutyric acid) is the primary inhibitory neurotransmitter in the central nervous system (CNS). Altered levels of GABA are associated with a series of neurological and psychiatric disorders, such as epilepsy, depression and schizophrenia [1].

Obtaining an MRS spectrum of GABA is problematic for two reasons. Firstly, concentration of GABA is inherently low compared to other metabolites. Secondly, GABA’s peaks overlap with the signals from other metabolites, such as creatine and glutamate and are not visible in spectra generated by clinically standardized MRS sequences. A method employed to overcome this is spectral editing, during which a pulse sequence called MEGA-PRESS is used. In a MEGA-PRESS experiment, spectra collected during even acquisitions differs from odd spectra. Ideally, subtracting the average even spectrum from the average odd spectrum results in an edited spectrum of GABA. The complexity of this experiment lies in the fact that its individual steps, such as the choice of scanner parameters, signal acquisition, post-processing design, etc. all require careful planning and good knowledge of the subject in order to yield desirable results. The aim of the project is to develop a suitable acquisition method and a reliable post-processing routine leading to the detection and quantification of spectral GABA.
1.2 The basics of MRS

$^1$H MRS is a technique based upon magnetic properties of hydrogen nuclei. Spin angular momentum, $L$, of elementary particles is given by

$$ L = \frac{h}{2\pi} \sqrt{I(I+1)} $$  \hspace{1cm} (1.1)

where $h$ is the Planck’s constant and $I$ is the spin quantum number that is composed of discrete half-integer values, $I = 0, \frac{1}{2}, 1, \frac{3}{2}, \ldots$ for nuclei with odd mass numbers. For all elementary particles $I = \frac{1}{2}$. This is the case for a hydrogen nucleus, seeing that it consists of a single proton.

Angular momentum aligned with the z-axis $L_z$ is given by

$$ L_z = \frac{h}{2\pi} m $$ \hspace{1cm} (1.2)

where $m$ is the magnetic quantum number, $m=I, I-1, I-2, \ldots, -I$.

Spin-$\frac{1}{2}$ particles also have a magnetic moment $\mu = \gamma L$, where $\gamma$ is the gyromagnetic ratio, a constant equal to 42.58 MHz/Tesla for hydrogen protons. Combining this with equation 1.1 we get an expression for the longitudinal $z$-component of the magnetic moment:

$$ \mu_z = \gamma m \frac{h}{2\pi} $$ \hspace{1cm} (1.3)

When placed in an external magnetic field $B_0$ the hydrogen nuclei will acquire magnetic energy, $E = -\mu_z B_0 = -\gamma \frac{h}{2\pi} m B_0$. A spin-$\frac{1}{2}$ particle will thus acquire one of two possible energy states, since $m = +\frac{1}{2}, -\frac{1}{2}$, where the difference between these energies is given by

$$ \Delta E = \gamma \cdot \frac{h}{2\pi} \cdot B_0 $$ \hspace{1cm} (1.4)

These states are not populated evenly, their ratio follows the Boltzmann distribution

$$ \frac{N_\alpha}{N_\beta} = e^{E_\beta - E_\alpha \frac{k}{\gamma}} $$ \hspace{1cm} (1.5)

where $N_\alpha$ represents the number of spins that align themselves parallel to the magnetic field and are the low energy spin-up state $\alpha$, and $N_\beta$ stands for the
1.2. THE BASICS OF MRS

number of spins that possess the high energy spin-down anti-parallel alignment ($\beta$ state). The intensity of the MR signal is proportional to the ratio of the number of protons in $\alpha$ and $\beta$ states.

1.2.1 Bulk magnetization and relaxation

In the absence of a magnetic field, the spins are oriented randomly and the macroscopic net magnetization is zero. Applying the magnetic field $B_0$ leads to the appearance of bulk magnetization. This is a result of the slight excess in the number of spins in the lower energy $\alpha$ state compared to the number of spins in the $\beta$ state. This bulk magnetization $M$ is aligned along the direction of $B_0$.

Resonance condition is achieved when an electromagnetic field is applied perpendicularly to $\mu_z$, to match the magnetic energy $\Delta E$ (eq. 1.4). The energy of this radiation lies within radio frequency (RF) range and is given by

$$\Delta E = h\nu_0$$

Combining equations 1.4 and 1.6 yields the expression for Larmor frequency, the characteristic precession frequency of nuclei placed in a magnetic field,

$$\omega_0 = \gamma B_0$$

The $90^\circ$ pulse tips the magnetization vector from being aligned along the $z$-axis onto the xy plane. Maximum signal is obtained when the entire magnetization vector lies in the xy-plane: $M_{\text{total}} = M_{\text{transverse}}$. After the RF transmission is stopped, relaxation begins. The magnetization vector again splits into a transverse and a longitudinal component, where the transverse magnetization decays and the longitudinal magnetization recovers over time. The measure of the recovery of longitudinal magnetization is described by the time $T_1$, which tells us the time when $M_z = 63\% M_{\text{total}}$. The type of relaxation described by $T_1$ is called spin-lattice relaxation. The general expression for longitudinal relaxation is

$$M_z(t) = M_z(t_0)(1 - e^{-t/T_1}) + M_z(0+)e^{-t/T_1}$$

where the term $M_z(0+)$ appears when the RF pulse does not tip the magnetization vector exactly $90^\circ$. 
Transverse magnetization can be described as follows

\[ M_{x',y'}(t) = M_{x',y'}(t_0)(e^{-t/T_2}) \]  

(1.9)

the term \( M_{x',y'}(t_0) \) denotes the transverse magnetization right after the RF pulse.

Besides the magnetization vector's gradual realignment with the z-axis, there is another process influencing \( M_{\text{total}} \) that is taking place at the same time. All the spins in a system do not precess at exactly the same frequency and their phase coherence decreases with time. The spins exchange energy between themselves, a process which gradually reduces the overall transverse magnetization. This loss of signal due to spin-spin relaxation is described by the time \( T_2 \) [2]. Another time constant which is often used in the context of spin-spin relaxation is the \( T_2^* \), which incorporates the effects of both spin dephasing and local magnetic field inhomogeneities.

### 1.2.2 Chemical shift

The resonance frequency of different nuclei varies slightly depending on their chemical environment. The electrons which surround the nuclei rotate in the opposite direction to the protons and thus shield them from the external magnetic field. This shielding allows one not only to recognize that there are hydrogen atoms present in the sample, but also to interpret their local environment and determine the molecule that contains them.

This new characteristic frequency is given by \( \omega_s = \gamma B_0(1 - \sigma) \), where \( \sigma \) is the shielding constant and \( B_0(1 - \sigma) \) amounts to the effective magnetic field. The frequency shift between nuclei in different chemical environment increases with an increasing magnetic field. It is to avoid this \( B_0 \) dependence that in MRS the spectra are not presented using a frequency axis, but are described using chemical shift \( \delta \), a quantity measured in ppm.

\[ \delta_{\text{chem.shift}} = \frac{\omega_s - \omega_{\text{ref}}}{\omega_{\text{ref}}} \cdot 10^6 \]  

(1.10)

where \( \omega_s \) is the frequency of the sample and \( \omega_{\text{ref}} \) is the reference frequency.
1.2. THE BASICS OF MRS

1.2.3 J-coupling

A feature common to most MR spectra is the splitting of resonance peaks into multiplets. This phenomenon occurs in systems that contain more than one spin-$\frac{1}{2}$ nucleus, and is called J coupling. J-coupling is a type of spin-spin interaction that is mediated by shared valence electrons and therefore occurs when two nuclei are close to each other in the same molecule.

![Diagram showing J-coupling](image)

**Figure 1.1.** A) In isolated atoms Fermi contact governs the orientation of the nuclear and electronic spins. In this case it is energetically favorable that the nucleus and the electron are anti-parallel. In atoms that are covalently bonded, the two electrons in the bond must be anti-parallel, even if this leads to an increase in energy. This is a consequence of the Pauli exclusion principle. B) When the atoms form a bond the $E_{A\beta B\beta}$ interaction is forced into an energetically less favorable state, hence the increase in energy. The $E_{A\beta B\alpha}$, in turn, decreases in energy since the electrons can be both anti-parallel to each other and to their respective nuclei.

When two isolated atoms A and B have their nuclear spins oriented along the -z axis ($\beta$-$\beta$ state), their electrons tend to align themselves along the +z axis to obtain the most favorable energy state. This happens as a result of the Fermi contact, which makes anti-parallel orientation between nuclear and electronic spins preferable. In the case of two covalently bound atoms, the situation is different, since their bonded electrons are now governed by the...
Pauli principle, meaning that the spin orientation of their respective electrons must be anti-parallel (fig. 1.1 (A))

The energy $E_{A\beta B\beta}$ is forced to increase with $\frac{h J_{AB}}{4}$, where $J_{AB}$ is the coupling constant, at the same time that the energy $E_{A\alpha B\alpha}$ decreases with the same amount (fig. 1.1 (B)), resulting in

$$\Delta E_{A(B=\beta)} = E_{A\beta B\beta} - E_{A\alpha B\beta} = h(\nu_A + \frac{J_{AB}}{2})$$

and

$$\Delta E_{A(B=\alpha)} = E_{A\beta B\alpha} - E_{A\alpha B\alpha} = h(\nu_A - \frac{J_{AB}}{2})$$

Therefore the resonance peak $\nu_A$ will be split into two peaks of equal height, one for each possible orientation of spin B (see fig. 1.2).

J-coupling is particularly interesting in the case of GABA, since it allows for spectral editing, making it possible to measure in vivo GABA concentrations.
1.3 SINGLE VOXEL SPECTROSCOPY

1.3 Single voxel spectroscopy

Single voxel spectroscopy (SVS) allows for signal acquisition from a single voxel in the brain. This restriction of a particular volume of interest (VOI) is made possible by varying the static magnetic field linearly, i.e. applying a magnetic field gradient. If a gradient in the $x$ direction ($G_x$) is applied, the total magnetic field experienced by a nucleus becomes dependent on its location along the $x$-axis:

$$B(x) = B_0 + xG_x$$ (1.11)

Combing equations 1.7 and 1.11 the Larmor frequency becomes position-dependent:

$$\omega = \gamma(B_0 + xG_x)$$ (1.12)

In clinical studies, the knowledge of the received frequency and the applied gradients allow for interpreting where in the patient the incoming signals originate. In SVS three mutually orthogonal slice selective gradient pulses are applied to restrict the excitation volume to a particular voxel.

1.3.1 PRESS

One of the most common spectroscopy acquisition sequences is the Point-RESolved Spectroscopy (PRESS). This sequence consists of three RF pulses of $90^\circ$, $180^\circ$ and $180^\circ$, each coinciding with three slice selective gradients along the $x$, $y$ and $z$ axis. This arrangement ensures that only the protons within the selected voxel experience excitation (fig. 1.3 (A)).

In a PRESS sequence a $90^\circ$ pulse is followed by a $180^\circ$ pulse, which is applied at a time $TE/4$ and has the effect of flipping the spins $180^\circ$ about the $y$-axis. This causes the spins that were lagging to now lead and vice versa. However, since the frequencies of the spins have not changed, the spins that end up behind eventually ‘catch up’ and at a time $TE/2$ all the spins are in phase again and a spin echo is produced. The second $180^\circ$ pulse is applied together with a $z$-gradient, which excites the spins in the $xy$-plane. The first echo is refocused by this pulse and the second echo is recorded as the signal at time $TE$. A detailed scheme of the PRESS sequence is presented in figure 1.5.
1.3.2 Shimming

In order to improve the signal-to-noise ratio (SNR) and to minimize the full width half-maximum (FWHM) values of the spectral resonances, the magnetic field over the VOI needs to be as homogeneous as possible. The process of fine-tuning the magnetic field over the chosen voxel is called shimming. Manual shimming during in-vivo studies can be a time-consuming project, however it is usually worth the trouble since insufficient shimming can result in poor spectral resolution. Proper shimming is what mainly governs the $T_2$ relaxation time constant.

1.3.3 Water suppression

For an MRS experiment to be successful, it is critical to ensure good suppression of the water signal. In the brain, the concentration of water protons rests at 80 M, whereas other metabolites are found at levels that are nearly 10,000 lower, on the order of 10 mM [3]. In order to minimize the intense water signal, water saturation is employed. Prior to the start of the acquisition, a narrow radio frequency saturation band is applied at exactly the Larmor frequency of water. This pulse is followed by the so-called spoiler gradients, which dephase any remaining transverse magnetization and ensure that water gives no signal.
1.4. GABA

GABA is the principal inhibitory neurotransmitter in the brain. Altered levels of GABA have been detected in people suffering from various neurological and psychiatric disorders, such as epilepsy, schizophrenia and substance abuse [1].

1.4.1 GABA-mediated inhibition

Increasing amount of GABA at the synapse prevents the generation of the action potential which inhibits signal transmission between the neurons. GABA released into the synapse interacts with two types of receptors. GABA-A receptors (ligand-gated ion channels), when open increase inward Cl$^-\text{ conduction, which leads to rapid hyperpolarization of the membrane. GABA-B receptors (G protein-linked receptors) are responsible for slower signal inhibition by increasing the post-synaptic K$^+\text{ conduction. GABA is one of the substances responsible for the excitatory-inhibitory balance in the cerebral cortex. When this balance is perturbed seizures may occur [4]. This is why GABA is often studied in the context of epilepsy. Animal models of genetic and acquired epilepsy have shown abnormal GABAergic functions, GABA agonist drugs manifest anti-convulsive action, whereas GABA antagonists are proconvulsive [4].}

1.4.2 Spectral editing of GABA using MEGA-PRESS

The H-NMR spectrum of GABA presents with a triplet at 2.28 ppm given by the C-2 protons, another triplet at 3.01 ppm due to the C-4 protons and lastly a quintet due to the C-3 protons at 1.89 ppm [5]. Besides its inherent low concentrations, accurate detection of GABA is complicated by various signal overlaps. At 2.28 ppm GABA signal is obstructed by that of glutamate and glutamine, at 1.89 ppm by NAA and NAAG, whereas at 3.013 ppm there is a complete spectral overlap with creatine (fig. 1.4).

In order to obtain signal from GABA, a technique called J-difference editing is used. This technique makes use of the J-coupling between the GABA’s C-3 protons at 1.89 ppm and the C-4 protons obstructed by creatine at 3.01 ppm. During the scan two spectra are obtained. Throughout an acquisition, every even scan (edit off) works like a regular PRESS sequence, whereas during odd acquisitions (edit on) a narrow bandwidth RF pulse is applied to the resonance peak at 1.89 ppm.
CHAPTER 1. INTRODUCTION

Figure 1.4. A) A simulation was made using the VeSPA software to demonstrate that each resonance of GABA is obstructed [6]. This is why GABA is practically undetectable unless spectral editing is used. B) Molecular structure of γ-aminobutyric acid.

The pulse sequence that makes this possible is called MEGA-PRESS. The MEGA-PRESS pulse sequence was generated on site by incorporating two selective 180° MEGA pulses into the standard PRESS scheme. The two 180° selective inversion pulses were placed around the second 180° slice-selective pulse of the PRESS sequence (see figure 1.5)
To briefly explain spectral editing, we turn to the same example of a spin system as considered in section 1.2.3, that is, two weakly-coupled nuclei A and B. A non-selective pulse affects both nuclei. During edit off acquisitions the non-selective 180° PRESS pulse has two effects on each spin present in the system. Firstly, it resets the phases so that \( \phi = \pi (\nu_A \pm J_{AB})TE \) becomes \( \phi = -\pi (\nu_A \pm J_{AB})TE \). Secondly, it reverses the direction of the rotation of the magnetic moments in the \( x'y' \) plane, which in a PRESS sequence leads to refocusing of the magnetic moment vectors at time TE/2.

A selective RF pulse will only affect the resonances that fall within its frequency range. In our case the narrow selective pulse was placed at 1.8 ppm, thus affecting the 1.89 ppm GABA resonance. This pulse also dephases the magnetic moments responsible for the 2 ppm NAA resonance, which explains the absence of the NAA peak in the edit on image in figure 1.6. As a result of the selective pulse, the C-3 hydrogen nuclei at 1.89 ppm undergo a population inversion. Due to the J-coupling between the 1.89 ppm GABA spins (B spins) and the 3.01 ppm GABA spins (A spins) the B spin population inversion manifests itself as the reversal of outer peaks of the C-4 resonance triplet shown in figure 1.6(B).
Figure 1.6. The spectrum was acquired from a brain phantom with increased GABA concentration. A) Selective refocusing. The narrow RF pulse is applied during the even edit on acquisitions. The pulse completely eliminates the NAA peak. B) Non-selective refocusing. The GABA triplet is not refocused, so the outer peaks are pointing downwards. This spectrum is what a PRESS-generated spectrum would look like. C) The edited spectrum. Clearly visible the edited GABA and Glu.

Subtraction of the two spectra allows observation of the outer lines of the GABA triplets and completely eliminates the creatine peak and all the other uncoupled resonances, since they are not affected by the narrow selective inversion pulse (figure 1.6(C)).
Chapter 2

Experimental

2.1 Signal acquisition with MEGA-PRESS

All experiments were conducted on a 3 Tesla Siemens Trio full-body MR scanner at Karolinska University Hospital, using a 32-channel receiver head-coil. The RF pulse frequency fed to the scanner is given as the number of ppm relative to the water resonance signal at 4.7 ppm. So for edit on acquisitions this value was set to 2.9 ppm, thus giving a pulse at 1.8 ppm. For the edit off scans, the pulse was applied symmetrically to the other side of the water resonance peak at 7.6 ppm. The exact frequency of this symmetrical pulse is unimportant as long as it ends up being outside our region of interest. The reason the pulse is at all applied and not simply turned off is to minimize baseline artifacts in the edited spectra. The goal is to make the even and the odd numbered acquisitions occur under similar conditions, thus reducing the difference between them to the edited spectra of interest.

To improve the sensitivity of the scans, the homogeneity of the local magnetic field over the VOI needed to be enhanced. Each new voxel placement was succeeded by a shimming procedure, comprising an automated 3D shimming routine followed by manual shimming.

2.1.1 The FID signal

The signal detected directly in an H-MRS experiment is the free induction decay (FID). When an RF pulse is applied perpendicularly to the $B_0$ field, the magnetic moments tilt away from the main magnetic field. When the pulse is no longer applied, the transverse magnetization of the nuclei starts to decay with a characteristic time $T_2^*$. As the spins realign with $B_0$ they lose energy, which gives rise to the FID signal.
The detector is built in such a way that it separately detects signal projection on the \( M_x \)-plane, which constitutes the real part of the FID:

\[
M_x(t) = M_0 (\cos(\omega_0 - \omega)t + \phi) e^{-t/T_2^*} \tag{2.1}
\]

and signal projection on the \( M_y \)-plane, which constitutes the imaginary part of the FID:

\[
M_y(t) = M_0 (\sin(\omega_0 - \omega)t + \phi) e^{-t/T_2^*} \tag{2.2}
\]

In equations 2.1 and 2.2, \( \phi \) is the phase at \( t = 0 \), \( \omega \) is the frequency of the RF pulse and \( \omega_0 \) is the Larmor frequency.

When the complex time-dependent signal is Fourier-transformed the real and the imaginary parts of the frequency-dependent signal are given by:

\[
R(\omega) = A(\omega) \cos(\phi) - D(\omega) \sin(\phi) \tag{2.3}
\]

\[
I(\omega) = A(\omega) \sin(\phi) + D(\omega) \cos(\phi) \tag{2.4}
\]

where \( A(\omega) \) and \( D(\omega) \) are the absorptive and dispersive Lorentzians, expressed by:

\[
A(\Delta\omega) = \frac{M_0/T_2^*}{(1/T_2^*)^2 + (\Delta\omega)^2} \tag{2.5}
\]

\[
D(\Delta\omega) = \frac{\Delta\omega M_0}{(1/T_2^*)^2 + (\Delta\omega)^2} \tag{2.6}
\]

From equations 2.3 and 2.4 it can be seen that when \( \phi = 0 \), the real spectrum is purely absorptive, whereas the imaginary spectrum is purely dispersive. However, when \( \phi \neq 0 \), the phase needs to be corrected by establishing the right linear combination of the real and the imaginary parts of the spectrum. This process, referred to as 'phasing' is an essential part of post-processing.

### 2.1.2 Processing in MATLAB®

A MATLAB® script was written to process the raw data files. The scanner output is in the DICOM format, so a tool called `dicom-open` and `dicom-get-spectrum-siemens` forming part of the DICOM Utilities [7] toolbox was incorporated into the script to allow reading the raw data.

The Image Processing Toolbox in MATLAB contains a number of tools that are capable of dealing with the DICOM format, amongst them the `dicominfo`
2.1. SIGNAL ACQUISITION WITH MEGA-PRESS

which reads the DICOM metadata and the *dicomread*, which opens dicom images. Unfortunately, none of these inbuilt MATLAB tools open DICOM spectra, which is why some tools from *DICOM Utilities* were used.

Based on the acquisition number the files were sorted into *edit on* and *edit off* series, for each of which an average was taken. The *edit on* and *edit off* series were then subtracted to give the edited spectrum. The time domain spectrum was zero-filled and apodized, followed by the implementation of the *fft* fourier transform, after which a manual phase correction was performed where necessary.

A customary part of processing is baseline correction. However, since the edited spectrum is a difference between the odd and the even acquisitions performed in identical conditions (apart from the narrow RF pulse during the even takes), any significant inclination of the baseline disappears with the subtraction of the two.

**Zero-filling**

Zero-filling is a post-processing step that is often used in MRS to improve the resolution. Zeros are added at the end of the FID signal, usually doubling the data vector. Zero-filling the time domain data corresponds to inserting extra frequency values between the existing points in the frequency axis. This step does not increase the amount of acquired data but it makes for a smoother looking spectrum. It is important to zero-fill a signal, which has completely decayed to zero, since otherwise a step will be created between the last data point and the first zero. This step function will then cause wiggles (characteristic of the sinc function) in the frequency spectrum. In our case, 1024 data points were zero-filled up to 2048 points in the time domain.

**Apodization**

Apodization is a way of weighing time domain data. Multiplying the FID with an apodization filter that amplifies the first part of the signal and suppresses the end of it (i.e. an exponential), will improve the SNR in the final frequency spectrum. However this will also give rise to line-broadening and therefore a poorer resolution. On the other hand, using a filter that amplifies the end of the FID will improve the resolution but increase the noise. Using an apodizing filter is a good way to bring the FID down to zero so as to avoid the wiggling artifact mentioned earlier as well as to create an optimal balance between resolution and SNR.
In our case a Gaussian apodization filter was employed, since it causes less broadening of the peaks at the baseline. This lack of longer ‘tails’ characteristic to Lorentzian shapes, is advantageous since it reduces the amount of spectral overlap, facilitating the quantification process.

2.2 Phantom study

The phantom study was conducted to be able to gain an understanding of how different parameters affect the obtained GABA signal. Conducting the experiment using different TE times showed that the time TE = 72 ms in combination with FQ = 2.9 ppm and BW = 0.9 ppm gave good results at TR = 2500 ms. FQ is the parameter, which determines the frequency of the applied selective pulse. The value is input into the scanner relative the water resonance at 4.7 ppm, in our case resulting in the frequency of 1.8 ppm, which is where the C-3 GABA and the NAA resonances are found. BW is the bandwidth of the selective pulse.

Since the concentration of NAA in the phantom is known, we could attempt a qualitative assessment of GABA, measuring the area under the edited GABA triplet relative to the area under the NAA peak. This would give us an idea about the potential yield of further measurements.

The phantom used in the study was a standard General Electrics brain phantom. The concentrations of metabolites present in the phantom are given in table 2.1. The original phantom did not contain GABA. We decided to raise the phantom GABA concentration to double the amount in healthy adult brain bringing it up to \( \sim 4 \text{ mM} \).

GABA (\( \geq 99\% \gamma\)-aminobutyric acid, Sigma Aldrich) was dissolved in a pH 7.2 phosphate buffer and injected into the phantom through a hole, which was drilled prior to the experiment. The resulting GABA concentration lay at \( \sim 3.9 \text{ mM} \). Several measurements were carried out at this concentration until it was doubled once more in order to study the ability of the method for detecting concentration change.
2.2. PHANTOM STUDY

Table 2.1. GE standard brain phantom. *The phantom with the 3.9 mM GABA concentration is further on referred to as the 2× phantom. **The second phantom containing 7.8 mM of GABA is the 4× phantom.

<table>
<thead>
<tr>
<th>Concentration</th>
<th>Abbreviation</th>
<th>Chemical Name</th>
</tr>
</thead>
<tbody>
<tr>
<td>50 mM</td>
<td>KH₂PO₄</td>
<td>Potassium phosphate monobasic</td>
</tr>
<tr>
<td>56 mM</td>
<td>NaOH</td>
<td>Sodium hydroxide</td>
</tr>
<tr>
<td>12.5 mM</td>
<td>NAA</td>
<td>N-acetyl-L-aspartic acid</td>
</tr>
<tr>
<td>10 mM</td>
<td>Cr</td>
<td>Creatine hydrate</td>
</tr>
<tr>
<td>3 mM</td>
<td>Ch</td>
<td>Cholnic chloride</td>
</tr>
<tr>
<td>7.5 mM</td>
<td>mI</td>
<td>Myo-inisitol</td>
</tr>
<tr>
<td>12.5 mM</td>
<td>Glu</td>
<td>L-glutamic acid (monosodium salt)</td>
</tr>
<tr>
<td>5 mM</td>
<td>Lac</td>
<td>DL-lactic acid (lithium salt)</td>
</tr>
<tr>
<td>3.9 mM</td>
<td>GABA (2×)*</td>
<td>γ-aminobutyric acid</td>
</tr>
<tr>
<td>7.8 mM</td>
<td>GABA (4×)**</td>
<td></td>
</tr>
</tbody>
</table>

2.2.1 Attempt at signal quantification using LCModel

Before subjecting the phantom to the MEGA-PRESS sequence, it was attempted to study the GABA content with the conventional PRESS sequence, which allows for analysis using LCModel [8]. This software permits quantification of in-vivo ¹H-MRS spectra. LCModel uses ready-made basis sets for combinations of different metabolites at certain TE. The concentrations present in the sample are estimated based on the proportion of the model spectra found in the experimental data. Unfortunately, LCModel does not have a basis set for MEGA-PRESS, so when using this tool we had to revert to the non-editing PRESS sequence.

The metabolite concentrations inside the phantom were compared to the LCmodel output. Due to the accurate detection of the amount of NAA (SD=3) the various concentrations were assessed relative this metabolite. It was found that LCmodel gives a reasonable prediction for concentrations of certain metabolites. However, it is not able to reliably assess the GABA concentration.

The glutamate concentration as measured by LCmodel lay at about half the actual value. This is due to the fact that Glu is obstructed in a similar way to GABA (see fig. 1.4) and also requires spectral editing for reliable quantification.
Figure 2.1. An example of LCmodel output for the 4x phantom (voxel size $= 15 \times 15 \times 15 \text{ mm}^3$). The estimated concentrations with respective standard deviations are shown on the right. On top of the spectrum the residuals are plotted.

Table 2.2. Actual metabolite concentrations compared to the concentrations estimated by LCmodel as a result of running the PRESS sequence. All concentrations relative given relative NAA.

<table>
<thead>
<tr>
<th></th>
<th>NAA</th>
<th>Glu</th>
<th>Lac</th>
<th>ml</th>
<th>Cho</th>
<th>GABA</th>
<th>Cre</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actual conc.</td>
<td>1</td>
<td>1</td>
<td>0.40</td>
<td>0.60</td>
<td>0.24</td>
<td>0.62</td>
<td>0.80</td>
</tr>
<tr>
<td>Estimated conc.</td>
<td>1</td>
<td>0.49</td>
<td>0.38</td>
<td>N/A</td>
<td>0.24</td>
<td>N/A</td>
<td>0.82</td>
</tr>
</tbody>
</table>

2.2.2 Signal quantification using MEGA-PRESS

Absolute quantification of metabolite concentrations in H-MRS spectroscopy is extremely difficult and often unreliable. This is why relative measurements are normally used. The variable directly proportional to metabolite concentration is the area under the peak assigned to this metabolite. In clinical studies the peak that is known to be unaffected by the disease is chosen as reference for the remaining metabolite concentrations. Conventionally, this unaffected compound is creatine, a chemical involved in energy metabolism. However the use of creatine as a reference is now being debated as decreased
2.2. PHANTOM STUDY

levels have been observed in e.g. tumors and stroke [9].

Naturally, MEGA-PRESS does not permit the use of creatine as a reference, as this peak is eliminated in the edited spectrum. So for this sequence we turn to trying to measure the area under the peaks of the edited spectrum as accurately as possible to later compare them to the NAA peak. Precise measurement of area under the peak is not always easy. Due to spectral overlap it is necessary to fit a mixture of Gaussians and Lorentzians to the individual peaks in order to deconvolve them. In our project this was done using *peaktool*, part of the MATLAB-NMR-Library [10].

![Figure 2.2](image)

**Figure 2.2.** An example of the *peaktool* function. This tool allows one to choose to fit only some peaks and not others, which is convenient as the only metabolites of interest in our case were GABA, Glu and NAA.
There are a number of different tools that can be used for signal quantification. One of them is LCModel with an appropriate basis set. The other is the AMARES algorithm implemented in the jMRUI software [11]. This tool uses prior knowledge, allowing the user to put various constraints on the parameters, like fixing the linewidths, the amplitude ratio between any two metabolites, etc. AMARES is a sophisticated method that allows one to use all the information available. However, creating a good estimate involves managing numerous parameters, which is cumbersome and time-consuming.

Since we are dealing with the edited spectrum of GABA, we do not need to deconvolve the peaks and it suffices to simply fit Gaussian/Lorentzian curves to individual peaks using peaktool (see figure 2.2).

The obtained areas under the peaks need to be normalized to the number of protons in the resonance group. The GABA triplet is generated by a CH$_2$ group, the NAA and the Glu resonances are due to the CH and the CH$_3$ groups respectively.

Another effect that needs to be accounted for when attempting metabolite quantification, namely, the time limitations of $TE$ and $TR$. In the theoretically ideal experiment, $TE = 0$ and $TR = \infty$. $TE = 0$ means that the signal is collected directly upon acquisition, ensuring no transverse dephasing of the spins. $TR = \infty$ implies that all the spins have had enough time to complete their longitudinal relaxation and are aligned with the z-axis before the next RF pulse is applied. Since this is not the case, corrections must be applied to the measured peak areas. For a PRESS sequence the corrected areas are given by:

$$\text{area}_{\text{corrected}} = \frac{\text{area}_{\text{measured}}}{e^{TE/T_2}(1 - e^{-TR/T_1})}$$

(2.7)

<table>
<thead>
<tr>
<th></th>
<th>Glutamate</th>
<th>NAA</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_1$(ms)</td>
<td>1200</td>
<td>1300</td>
<td>1100</td>
</tr>
<tr>
<td>$T_2$(ms)</td>
<td>200</td>
<td>300</td>
<td>100</td>
</tr>
</tbody>
</table>

Table 2.3. $T_1$ and $T_2$ values for NAA, Glu and GABA.
2.2. PHANTOM STUDY

The phantom experiments were carried out at $TE = 72$ ms and $TR = 2500$ ms. The $T_1$ and $T_2$ for the metabolites in question vary depending on the source, some authors give separate values for gray and white matter, in this case a mean value of the two was used for the phantom studies. The $T_1$ for GABA is not known and was assumed to be 1100 ms [12].

The duration of the scan is insignificant during phantom measurements but it can be crucial when scanning patients. Sets of measurements of different lengths were carried out so as to decide upon an adequate length for a scan, which would still give an acceptable signal to noise ratio.

Figure 2.3. Spectra from 2× GABA phantom shows the signal collected using a different number of acquisitions. The difference between the signal acquired using 256 meas. and 512 meas. is apparently small, so it might be suggested that in clinical studies where acquisition time is of great importance, 256 measurements suffice.
Table 2.4. Quantification of GABA relative to NAA. Here compared to the the known metabolite ratio inside the $2 \times$ GABA phantom.

<table>
<thead>
<tr>
<th>Actual ratio</th>
<th>NAA</th>
<th>Glu</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>128 meas. (5.20 min)</td>
<td>1</td>
<td>1</td>
<td>0.31</td>
</tr>
<tr>
<td>256 meas. (10.40 min)</td>
<td>1</td>
<td>0.46</td>
<td>0.15</td>
</tr>
<tr>
<td>512 meas. (21.20 min)</td>
<td>1</td>
<td>0.74</td>
<td>0.15</td>
</tr>
</tbody>
</table>

After a number of experiments, the concentration of GABA inside the phantom was doubled and now amounted to four times the concentration in a healthy adult brain. It was interesting to investigate the effect the voxel size has on the amount of acquired signal. A small voxel has the advantage when it comes to shimming, since a homogeneous magnetic field is much easier to acquire over a small volume.

Figure 2.4. Spectra from $4 \times$ GABA phantom shows the signal collected from voxels of different sizes.

Judging from these spectra one can suggest that for a phantom study, where the noise levels are low and the baseline is straight even the smallest voxel size

22
2.2. PHANTOM STUDY

suffices, however a phantom signal yield can never be obtained from a living subject, so a large voxel is preferable for better SNR (equation 2.8).

Table 2.5. Quantification of GABA relative to NAA for different-sized voxels. Here compared to the the known metabolite ratio inside the 4× GABA phantom.

<table>
<thead>
<tr>
<th>Actual ratio</th>
<th>NAA</th>
<th>Glu</th>
<th>GABA</th>
</tr>
</thead>
<tbody>
<tr>
<td>15×15×15 mm³</td>
<td>1</td>
<td>1</td>
<td>0.62</td>
</tr>
<tr>
<td>20×20×20 mm³</td>
<td>1</td>
<td>0.40</td>
<td>0.29</td>
</tr>
<tr>
<td>30×30×30 mm³</td>
<td>1</td>
<td>0.63</td>
<td>0.30</td>
</tr>
</tbody>
</table>

The goal of the phantom study was to try and estimate the amount of GABA relative to the known amount of NAA and see how it compares to the actual quantity of GABA present in the phantom. Going from the 2× phantom to the 4× phantom allowed us to assess how well our method detects this change in GABA concentration.

The results of the 2× phantom and the 4× phantom measurements are presented in tables 2.4 and 2.5. They show that peaktool is able to demonstrate the twofold increase in GABA concentration. However, the absolute signal yield as estimated by peaktool amounts to only about 50% of the theoretically available signal.
2.3 In-vivo study

The subject group consisted of 8 healthy volunteers (6 male and 2 female) all of whom were informed about the purpose of the study. One of the tasks of the in-vivo experiment was to study the effect of various parameters, such as voxel size and location, acquisition time, homogeneity of the $B_0$ field etc. on the quality of the signal.

Before each MEGA-PRESS pulse sequence, 15 - 20 slices were acquired in the sagittal, transverse and coronal planes, to allow for optimal placement of the voxel. This was done using the $T_2$-weighted HASTE sequence ($TE = 81$ ms, $TR = 2000$ ms). The acquisition lasted for $\sim 1.50$ min.

2.3.1 Voxel placement

GABA levels in the brain differ between gray and white matter areas. A nearly twofold increase in GABA levels in cortical gray matter is revealed compared to white matter [13]. Initially our goal was to separate the gray and the white matter signal by placing the voxel so that it contained mainly only one of the substances. This type of separation proved challenging to perform. Placing the voxel on the border of the two hemispheres and acquiring signal from predominantly the gray matter was quite straightforward. Attempting to select volumes of mainly white matter was more problematic and the majority of the signals gained from these attempts were contaminated as a result of the voxel’s close proximity to CSF, lipids or skull bone. Therefore the idea of rigorous separation of the two substances was abandoned in favour of trying to collect stronger signal from mixed voxels instead. Figures 2.5 - 2.7 demonstrate different voxel placements and some typical outcomes of edited spectra.

To gain substantial signal from in-vivo acquisitions, the voxel volume was chosen to be $\sim 27\text{mL}$. In MRS, the signal increases proportionally to the volume of the pixel [14], whereas the noise decreases with the square root of the total number of measurements $N$, yielding the SNR dependence:

$$SNR \propto v_x v_y v_z \sqrt{N}$$

(2.8)
2.3. IN-VIVO STUDY

**Figure 2.5.** An example of a voxel placement for investigating gray matter. It is appropriate to try and place the voxel at the fold between the two hemispheres, so as to include as much of the cerebral cortex as possible, gaining the signal from predominantly gray matter. Voxel size $40 \times 25 \times 30 \text{ mm}^3$.

**Figure 2.6.** Edited GABA spectrum gathered from the volume presented in figure 2.5 acquired from 256 measurements. TE = 72 ms, TR = 2500 ms. The line-width after shimming lay at 12.2 Hz.
Figure 2.7. An example of a GABA study with MEGA-PRESS at 3T. A) A 30×30×30 mm$^3$ voxel located in the parietal lobe. It is placed so that a maximum amount of the cerebral cortex is included. B) A 40×25×30 mm$^3$ voxel placed in the frontal lobe. The selected volume is located in the left hemisphere and includes both gray and white matter. Fat saturation bands were added so as to reduce the signal from the surrounding lipids. C) Resulting spectra after three acquisitions consisting of 512 measurements each. i. A spectrum obtained from voxel A, where a characteristic doublet-like shape of the edited GABA is visible. Line-width after shimming: 11.5 Hz. Spectra ii. and iii. are generated from the signal of voxel B. Because of the inhomogeneity of the selected volume the quality of the spectra is poorer. This is also the reason for worse shimming results, the best line-width value after shimming lay at 13 Hz.
2.3. IN-VIVO STUDY

2.3.2 In-vivo signal quantification

In order to quantify the results of the in-vivo experiments, peaktool was used again to estimate metabolite concentrations. The challenge of in-vivo experiments is absence of knowledge about actual metabolite concentration as well as high inter-subject variability, arising mostly from voxel composition but also from gender effects, caffeine levels etc. [15]

Figure 2.8 presents the obtained concentrations of GABA and Glx (glutamate and glutamine) relative to the NAA signal. The resulting mean ratios are mean$_{GABA/NAA}$ = 0.24, SD = 0.05 and mean$_{GABA/Glx}$ = 0.50, SD = 0.09. It would be interesting to further compare these ratios obtained from healthy controls to the same ratios obtained from patients with disorders, involving changes in GABA and NAA levels. Since quantification of absolute metabolite concentrations is not possible, a future investigation should be to determine whether there exists a correlation between the GABA/NAA ratio in patients and in controls.

Another interesting aspect of the in-vivo experiments is concerned with the reproducibility of the results. In the in-vivo study one subject (number 3 in figure 2.8) was scanned on two different occasions, with the resulting ratios GABA/NAA=0.21 and GABA/NAA=0.22. Additional longitudinal studies involving scans and rescans of several subjects will provide reliable statistics regarding the reproducibility of the results.
Figure 2.8. Quantification of GABA relative to NAA for the in-vivo experiments. The obtained areas were corrected for the $T_1$ and $T_2$ values of the specific metabolites, as in the case of the phantom study. Subject 3 was scanned on two different occasions, hence the data marked with 3*. Subjects 4 and 5 are female, the rest are male. In subjects 2 and 5 the voxel was located in the parietal and the occipital lobes respectively. The rest of the voxels were located in the frontal lobe. In subjects 1 and 4, the voxels were placed entirely in one of the hemispheres. In the remaining cases we attempted to mainly gather the signal from gray matter, so the VOIs were placed on the border between the two hemispheres in order to maximize the volume of the cerebral cortex included.
Due to the involvement of $\gamma$-amino-butyric acid in a number of neurological disorders, there is little doubt about the clinical importance of successful detection of cerebral GABA. The quality of spectral editing depends on a number of factors. The routines employed by different research groups for signal acquisition and processing vary greatly. One general obstacle is the complicated nature of in-vivo experiments. There is little possibility to carry out two identical experiments or put constraints on parameters. The contents of the chosen voxel as well as voxel’s location within the brain have a profound effect on the inter-subject variability of SVS studies.

### 3.1 Choosing the appropriate RF pulse

#### 3.1.1 Sinc vs Gaussian

In order to achieve ideal selective excitation, a pulse is needed that will excite all the spins within a certain bandwidth and none outside of it. Theoretically, such a pulse is a sinc-pulse, since its Fourier transform is a rectangular function. However, to produce an ideal square profile, the sinc-shaped pulse would have to be infinite, which is not possible. So, the pulse is truncated, yielding a compromised slice profile, which is only approximately rectangular.

Selective excitation can also be achieved using a Gaussian pulse. This is convenient since a Gaussian in the time domain yields a Gaussian in the frequency domain when Fourier transformed. To create a narrow excitation profile, i.e. excite only a few selected frequencies, a long Gaussian pulse is needed. To excite a wider frequency range, a narrow pulse is used.
In the original MEGA-PRESS sequence [16] the pulses used for selective excitation are Gaussian-shaped (i.e. MEGA-pulses in fig. 1.5). However, in the MEGA-PRESS implemented in our project, sinc pulses were used instead. This may have had an affect on the outcome of the experiment. In order for them to be successful, sinc pulses must be truncated minimally and therefore tend to have longer durations than the Gaussian pulses. For example, if we were to carry out water suppression, by selectively exciting the water resonance at 250 Hz a Gaussian pulse would have to last for 10.8 ms whereas a sinc pulse would have to be applied for 23.9 ms [9]. Consequently, using the long sinc pulse for water suppression would lead to incomplete water suppression because of $T_1$ and $T_2$ relaxation. It is mentioned in several sources that under time constraints Gaussian pulses are superior to sinc pulses when it comes to exciting a narrow frequency band [17][9]. So in future experiments using Gaussian-shaped RF pulses might be considered.

During the MEGA-PRESS experiment, the RF pulse with a FWHM=0.9 ppm is applied at 1.8 ppm. This means that the ‘tail’ of the pulse stretches over to the GABA resonance at 2.3 ppm. It is desirable to make the pulse as narrow as possible, so as to be able to only affect the C-3 GABA resonance. However this would require for the pulse to be applied for a longer time, thus increasing the minimum TE. In most works which deal with the subject, TE values lay at 68 ms [18], 70 ms [19] or 72 ms [1]. We achieved good results using the TE = 72 ms. It was found that the difference in signal between the three echo times was negligible.

To determine the appropriate frequency at which to apply the selective MEGA pulse, several placements were attempted, before the frequency of 1.8 ppm was chosen. Our hypothesis was that the ‘tail’ of the selective pulse might affect the other resonances and it would be interesting to observe what happened if we moved the pulse further in the decreasing direction of the ppm scale. According to the results presented in figure 3.1, a successful evolution of the GABA triplet’s outer peaks is possible when the RF pulse is applied at 1.8 ppm and 1.7 ppm. Moving it further in the direction of decreasing chemical shift gives little signal.
3.2. EFFECTS OF MAGNETIC FIELD INHOMOGENEITY

3.2.1 Magnetic susceptibility

Magnetic induction $B$ felt by the nuclear moments depends on the magnetic susceptibility of the material as:

$$B = \mu_0(1 + \chi)H$$

where $\chi$ is the magnetic susceptibility, a property which indicates how readily a material gets magnetized as a response to an applied magnetic field. $H$ is the magnetic field strength and $\mu_0 = 4\pi \cdot 10^{-7}\text{Hm}^{-1}$ is the magnetic permeability in vacuum.

The perturbation of the magnetic field is most pronounced near the ventricles, due to the difference in magnetic susceptibility between CSF and tissue. High magnetic field inhomogeneity is thus found above the sinus cavity (frontal lobe) and near the auditory tracts (temporal lobe) [9]. Coincidentally, the frontal and the temporal lobes are of particular interest for partial epilepsy studies, since it is in these regions of the brain that most seizures originate.

3.2.2 The importance of shimming

The quality of the shimming has been crucial to the success of the scans. The smallest linewidths obtained lay at $\sim 3$ Hz for the phantom (fig. 2.4) and $\sim 12$ Hz (fig. 2.6) for the in-vivo study. Simulation in figure 3.2 gives a visual
representation of how different shimming results affect resolution. The success of the shimming is what accounts for the difference between the shape of the edited GABA in phantom and \textit{in-vivo}. Obtaining a homogeneous field over a chosen voxel was a challenge during the course of the \textit{in-vivo} study. Meticulous manual shimming can be rather time-consuming, which is a drawback in all \textit{in-vivo} experiments. We have seen that if the linewidth rests at \(\sim 18\) Hz or above after the automated 3D shim, it proves inefficient to try and shim it down to the desired \(\sim 12-13\) Hz manually. A better approach is to attempt a new 3D shim or to find a better voxel placement.

\begin{figure}[h]
\centering
\includegraphics[width=0.5\textwidth]{figure3.2.png}
\caption{GABA spectrum simulated using the VeSPA software [6]. Note that with the linewidth of 1.5 Hz, each peak of the GABA triplet is split into subsequent doublets, whereas at 9 Hz the triplet is completely unresolved.}
\end{figure}
3.2. EFFECTS OF MAGNETIC FIELD INHOMOGENEITY

Poor shimming can also lead to miscalculation (usually overestimation) of metabolite concentrations (figure 3.3). This is understandable, since when the peaks are poorly resolved their apparent area increases. Thus the fitting of Gaussian and Lorentzian curves becomes more problematic and the risk of overestimating the area under the curve increases.

**Figure 3.3.** Measured concentrations plotted against the linewidths acquired after the shimming. Green shapes represent GABA concentrations, blue shapes represent the amount of the Glx complex. It can be seen that there is a tendency to measure higher metabolite concentrations at higher linewidths. Same shapes in different colors stand for GABA and Glx values of the same subject. Subject 5 was not included in this analysis since the shimming values were not recorded.
3.3 The challenges of signal quantification

When MEGA-PRESS is implemented and a good post-processing routine has been developed, there remains a question of robust signal quantification. There are many ways in which quantification of GABA signal can be carried out. There have been studies where concentration was estimated relative the Cr peak [20], relative the unsuppressed water resonance [21] and relative NAA signal [22].

The three most common methods used for signal quantification are the jMRUI AMARES algorithm, a Matlab peak-fitting tool and the more advanced LCModel. LCModel has the advantage of being applicable to spectra with overlapping peaks. In our study the peaks in the edited GABA spectrum were deconvoluted, so we felt that using a MATLAB curve-fitting tool sufficed. Nevertheless, for future studies it would be interesting to create an LCModel basis set for evaluation of in-vivo GABA spectra. A new basis set can be simulated using the VeSPA software [6]. The main advantage of using LCModel would be its increased reliability, due to its 'black box' automated nature. Both AMARES and peaktool have the disadvantage of being more time-consuming and perhaps more importantly relying upon human input, which yields results that are dependent on the investigator.

3.4 Future applications

GABA MRS has a number of uses in clinical neuroscience. Among other potential prospects, there is a hope of obtaining new results while studying generalized epilepsy. In patients suffering from primary generalized epilepsy (PGE) abnormalities have been found in the thalamocortical network [23]. Ideally one would want to investigate GABA concentrations somewhere in this region. However, homogenizing the magnetic field over a VOI that is large enough to give good SNR might be challenging, because of the proximity of this region to ventriculus tertius and ventriculus lateralis.

It is also evident that for epilepsy studies quantification of GABA signal relative NAA would be inaccurate in some cases. It has been shown that juvenile myoclonic epilepsy (JME) patients have presented with decreased frontal lobe NAA levels compared to healthy controls, whereas patients suffering from generalized tonic clonic seizures (GTCS) have lower NAA levels in the thalamus [24]. However frontal lobe NAA levels for GTCS and thalamus NAA levels for JME are within normal interval [24]. Choline and myo-inisitol levels in
3.4. FUTURE APPLICATIONS

the thalamus were reduced for both JME and GTCS patients [24]. Due to the thalamus’ involvement in PGE, many metabolite levels are affected by the disease simultaneously, so there is no possibility to use one resonance as steady reference. One way to tackle this complication involves gathering information about different ratios, as mentioned in section 2.3.2. By scanning and rescanning both healthy controls and affected patients, it should be determined whether there is a pattern in the way the GABA/NAA, Glx/NAA, GABA/Glx ratios are altered depending on the particular disease condition.
List of abbreviations

BW Bandwidth  
CNS Central Nervous System  
Cr Creatine  
CSF Cerebrospinal fluid  
FID Free Induction Decay  
FWHM Full Width at Half Maximum  
GABA γ-aminobutyric acid  
Gln Glutamine  
Glu Glutamate  
Glx Glutamate and Glutamine  
GTCS Generalized Tonic Clonic Seizure  
JME Juvenile Myoclonic Epilepsy  
MRS Magnetic Resonance Spectroscopy  
NAA N-acetyl-aspartate  
PGE Primary Generalized Epilepsy  
PRESS Point-RESolves Spectroscopy  
RF Radio Frequency  
SNR Signal-to-Noise Ratio  
SVS Single Voxel Spectroscopy  
TE Echo Time  
TR Repetition Time  
VOI Volume Of Interest
Acknowledgments

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This research has made use of the SMILE medical imaging laboratory at Karolinska University Hospital and Karolinska Institute, Stockholm, Sweden.
Bibliography


[11] jMRUI version number 4.0: MRS processing software package developed by D. Graveron-Demilly group at the Claude Bernard University Lyon 1 in France.


