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Detection of the Excitatory Neurotransmitter Glutamate in the Human Brain Using Glutamate-CEST at Ultra-High Magnetic Field Strength (7 T)

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POPULÄRVETENSKAPLIG SAMMANFATTNING

DETEKTION AV SIGNALSUBSTANSEN GLUTAMAT I HJÄRNAN MED HJÄLP AV GLUTAMAT-CEST VID ULTRAHÖG MAGNETFÄLTSTYRKA (7 T)

Nerver i hjärnan kommunicerar med varandra och med andra celler genom att utsöndra ämnen, så kallade *signalsubstanser*, som agerar aktiverande eller hämmande beroende på sort. Den vanligaste aktiverande signalsubstansen i hjärnan är aminosyran glutamat (Glu), som även deltar i produktionen av den vanligaste hämmande signalsubstansen gamma-aminosmörsyra (GABA). Dysfunktion i produktion av Glu eller i en rubbad balans av GABA och Glu har kopplats till ett flertal neurologiska sjukdomar, exempelvis epilepsi, Alzheimers, Parkinsons, essentiell tremor och gliom (hjärntumör). Detektering av Glu i hjärnan bidrar till kunskapen om dessa sjukdomar och kan vara till hjälp vid beslut gällande diagnos eller behandling.

Tidigare icke-invasiv metod för detektering av Glu har varit magnetresonans-spektroskopi, som begränsats av lång insamlingsstid, relativt låg upplösning, och svårighet att urskilja glutamat från andra ämnen. En relativt ny teknik inom magnetresonansbildtagning (MRI), kallad CEST (Chemical Exchange Saturation Transfer), överkommer dessa begränsningar. CEST bygger på indirekt detektion av ett ämne, till exempel Glu, genom att utnyttja det naturliga utbytet av vätekärnor mellan ämnet och intilliggande vatten. Genom att skicka in en specifikt utformad radiofrekvent (RF) våg kan signalen från vätekärnor i Glu släckas ut, vilket benämns *saturation*. När dessa vätekärnor utbyts med vattenmolekyler följer saturationen med vilket leder till att den totala vattensignalen minskar. Den sänkta vattensignalen är bland annat proportionell med mängden Glu och kan därför skapa en bild med relativ mängd glutamat i hjärnan som kontrast.

För att detektera den sänkta vattensignalen orsakad av Glu behövs ett starkt externt magnetfält (B_0) och kräver att man samlar in en bild efter saturation med RF vågen för flera olika frekvenser. Bilderna normaliseras med avseende på en bild insamlad helt utan saturation, sedan studerar man signalen som en funktion av RF vågens frekvens, kallad *Z-spektrum*. Det starka magnetfältet B_0 är inte jämnt, utan har små variationer som orsakar att frekvensen som väteatomerna i Glu upplever är annorlunda från den RF våg som skickas in. Man kan korrigera för detta till viss del innan CEST bildtagningen, men små variationer kvarstår och behöver korrigeras för efteråt.

Detta projekt hade i syfte att genomföra CEST bildtagning av Glu, skapa en metod för korrektion av variationer i B_0 , samt att etablera ett protokoll på skannern för upprepbar CEST bildtagning. Till förfogande var Philips 7 T Achieva MRI skanner i Lund och bildtagning genomfördes med tre fantom (olika mängd glutamat och pH värde) samt med hjälp av tre friska frivilliga.

Hastigheten för utbytet av vätekärnor mellan Glu och vatten beror starkt på pH och mätning visade tydlig skillnad i signal mellan de tre fantomen. Styrkan och tidslängden av RF vågen påverkar också signalen vid CEST bildtagning och mätning av fantom visade en positiv ökning av signal för både ökad styrka och längre tid. Liknande positiv respons vid ökad RF styrka uppmättes i frisk frivillig och överensstämde med vad som var förväntat. Vid CEST bildtagning av friska frivilliga uppmättes ett starkt bidrag från saturation av andra väteatomer än de i Glu och som överför saturation till vatten genom andra processer än genom utbyte av vätekärnor. Dessa processer dominerar vid vissa frekvenser av RF vågen och leder till längre värden i CEST bilderna. Trots det detekterades en sänkt vattensignal vid frekvensen för vätekärnorna i Glu i Z-spektrumet. Dessutom visade CEST bilder en korrekt kontrast mellan vitsubstans och gråsubstans hos de tre friska frivilliga.

Det var möjligt att skapa en korrektionsmetod för variationer i B_0 och beräkning av CEST bilder i MATLAB, samt att skapa ett protokoll för CEST bildtagning av Glu på 7 T skannern. Trots att korrektion av variationer i B_0 var framgångsrik var det tydligt i CEST bilderna att variationer i själva RF vågen var stora och därav påverkade bilden kraftigt. Därför behövs en metod för korrektion av dessa variationer för framtida applikationer.

ABSTRACT

BACKGROUND AND PURPOSE

The amino acid glutamate (Glu) is the primary excitatory neurotransmitter in human brain and is involved in both synaptic transmission as well as metabolic pathways. Dysfunction of glutamate concentration in the brain has been implicated in neurological diseases, such as epilepsy, Alzheimer's disease, schizophrenia and essential tremor. Non-invasive detection of glutamate concentration using the novel MRI technique named chemical exchange saturation transfer (CEST) was demonstrated in 2012 and it displays a superior spatial resolution and signal-to-noise ratio (SNR) compared to standard proton magnetic resonance spectroscopy (^1H MRS). Glutamate-weighted CEST imaging require ultra-high magnetic field strength (≥ 7 T) and results have shown a high degree of reproducibility in healthy volunteers at 7 T.

The aim of this master's project is to implement GluCEST imaging at the National 7 T facility in Lund and to detect glutamate concentration in phantoms as well as in healthy volunteers.

THEORY AND METHODS

Glutamate is indirectly detected by saturating protons in the glutamate amino group with a frequency selective radiofrequency (RF) pulse, which then transfers to surrounding water through so called chemical exchange. The resulting decrease in water signal, the so called CEST effect, has a strong pH and RF pulse shape dependency.

A sequence designed for CEST imaging was modified under a research agreement on the Philips 7 T Achieva magnetic resonance imaging (MRI) system in Lund. The CEST effect detected by the sequence was investigated using three phantoms with different pH-values and Glu concentrations, as well as varying RF amplitudes and durations for each phantom.

Subsequently, three healthy volunteers were scanned using the RF pulse parameters that maximised CEST effect without exceeding specific absorption rate (SAR) restrictions, as determined from the phantom experiments.

RESULTS AND DISCUSSION

Phantom measurements displayed higher CEST effect at the resonance frequency of the glutamate amine (3 parts-per million (ppm) downfield from bulk water) for lower pH-values and a proportionality to RF amplitude and duration. These results concur with previous studies and indicate that Philips CEST sequence is able to perform GluCEST imaging on a 7 T MRI system.

Noticeable effect from radiofrequency magnetic field (B1) inhomogeneity was observed in GluCEST images, both in phantoms and healthy volunteers, highlighting the need for post-process B1 correction.

A strong contribution from nuclear Overhauser enhancement (nOe) was observed in healthy volunteers, resulting in images with negative values which was not expected. However, a CEST effect was observed at frequency 3 ppm downfield from bulk water and expected contrast between brain tissues were visible.

CONCLUSIONS

Measurements of Glu in phantoms and healthy volunteers were possible using Philips CEST sequence, but required robust post-processing for B0 and B1 inhomogeneities. For further work, developing a method for B1 correction is suggested and investigating the possibility of expanding single-slice into volumetric imaging is recommended for future applications.

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LIST OF ABBREVIATIONS

^1H MRS	Proton Magnetic Resonance Spectroscopy
APT	Amide Proton Transfer
B0	Static Magnetic Field
B1	Radiofrequency Magnetic Field
CEST	Chemical Exchange Saturation Transfer
DS	Direct Saturation
FSE	Fast Spin Echo
GABA	Gamma-aminobutyric acid
Gln	Glutamine
Glu	Glutamate
GluCEST	Glutamate-weighted Chemical Exchange Saturation Transfer
GM	Gray Matter
GRE	Gradient Echo
HC	Healthy Control Subject
MRI	Magnetic Resonance Imaging
MT	Magnetisation Transfer
MTR_{LD}	Linear Difference Magnetisation Transfer Ratio
MTR_{asym}	Asymmetric Magnetisation Transfer Ratio
nOe	Nuclear Overhauser Enhancement
PTR	Proton Transfer Ratio
RF	Radiofrequency
SAR	Specific Absorption Rate
SE	Spin Echo
SNR	Signal-to-Noise Ratio
ssMT	Semi-solid Magnetisation Transfer
WM	White Matter

1 INTRODUCTION

The amino acid glutamate (Glu) reside naturally in our central nervous system, acting as a so called excitatory neurotransmitter, a chemical released by neurons to signal actions to cells or other neurons [1]. Glutamate, together with the inhibitory neurotransmitter gamma-aminobutyric acid (GABA), makes up the two major neurotransmitters in the human brain, with Glu being the primary excitatory neurotransmitter. Detection of Glu *in vivo* has contributed to our knowledge of normal brain physiology and to the etiology of certain diseases, as well as help inform treatment approaches [2]. Furthermore, the dysfunction or abnormality in Glu concentration has been implicated in several neurological diseases, such as epilepsy [3], Alzheimer's disease [4], Schizophrenia [5], gliomas [6] and essential tremor [7].

Glutamate can be detected using proton magnetic resonance spectroscopy (^1H MRS), a technique that detect different metabolites in a volume based on information about the environment surrounding hydrogen atoms [8]. Glutamate has a complex spectral pattern with overlapping resonances from other metabolites, making the individual contribution of Glu difficult to identify *in vivo* [9]. Furthermore, ^1H MRS generally have long acquisition times in order to achieve a sufficient signal-to-noise ratio (SNR) as well as having poor spatial resolution (often around 8 cm^3 voxelsize), most often being single-voxel based [10].

Non-invasive detection of Glu concentration *in vivo* was previously possible through ^1H MRS, up until the introduction of Glu imaging using chemical exchange saturation transfer (CEST), so called GluCEST, in 2012 [11]. The principle of CEST is not new, techniques based on saturation transfer have been used for example in *in-vivo* studies using ^{31}P MR spectroscopy. However, utilising the effect on the ^1H signal, CEST allow for imaging rather than being limited to spectroscopy. This novel magnetic resonance imaging (MRI) technique can indirectly detect Glu through the chemical exchange of hydrogen nuclei between the amino group ($-\text{NH}_2$) in Glu and those in neighbouring water molecules [10]. A well-defined radiofrequency (RF) pulse can saturate only the exchangeable hydrogen nuclei in Glu, which are then transferred to water through chemical exchange, resulting in a decreased water signal. Chemical exchange saturation transfer imaging operates through continuous saturation of hydrogen nuclei in the solute of interest which then continuously exchange with those in water, leading to an accumulative saturation effect and thus increased sensitivity [12].

Highly reproducible measurements using GluCEST for healthy volunteers has been done using 7 T [13]. Based on Glu having relatively fast exchange rates, it is necessary to use high magnetic field strengths of 7 T or higher to observe the CEST effect from Glu [14]. Higher magnetic field strength entail improved SNR, contrast and spatial resolution but is also more susceptible to inhomogeneities in the static magnetic field (B_0) as well as the radiofrequency magnetic field (B_1), hence GluCEST imaging require corrections in regards to both [11]. Generally these corrections are done with the help of B_0 and B_1 fieldmaps, generated by a range of different methods either separately [15, 16, 17, 18, 19] or simultaneously [20].

The aim of this masters project is to implement GluCEST imaging capable of detecting Glu concentration, firstly in phantom measurements and secondly in healthy volunteers, using the National 7 T facilities MRI scanner in Lund.

2 THEORY

2.1 GLUTAMATE - THE MAIN EXCITATORY NEUROTRANSMITTER

Neurons signal each other or targeted cells through synaptic transmission, with the majority occurring through release of chemicals, named *neurotransmitters*. These neurotransmitters carry messages over synapses, the space between a neuron and another neuron or target cell, where the two primary neurotransmitters in the human brain are amino acids glutamate (Glu) and gamma-aminobutyric acid (GABA) [1].

Glutamate acts as the primary excitatory neurotransmitter and is naturally synthesised in the human body. Glutamate is produced in the transamination of α -ketoglutarate (Figure 1), a metabolite synthesised in the Krebs (citric acid) cycle [21]. Glutamate, together with the similar amino acid glutamine (Gln), is also part of the glutamate-glutamine cycle (Figure 2), an integral part in neuronal activity as well as regulation of Glu concentration in the central nervous system [2]. Glutamate is present in much higher concentrations in the human brain, than Gln, and primarily within neurons.

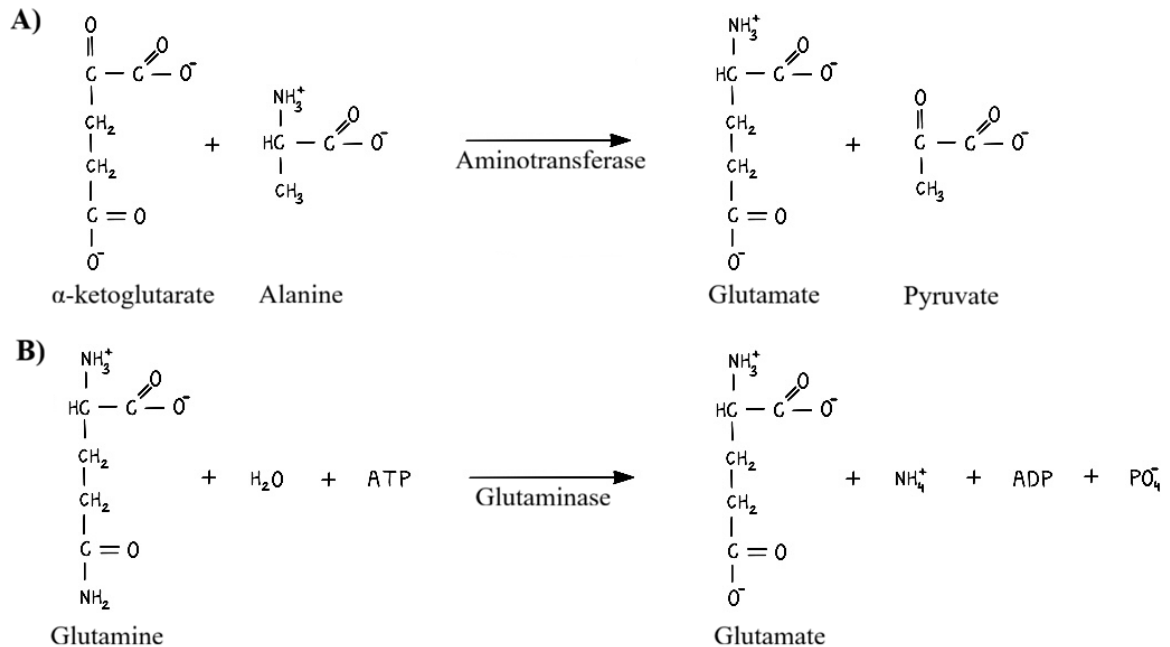


Figure 1: The two major synthesis pathways of glutamate: A) the conversion of α -ketoglutarate into glutamate through aminotransferase and B) the conversion of glutamine into glutamate by the enzyme glutaminase.

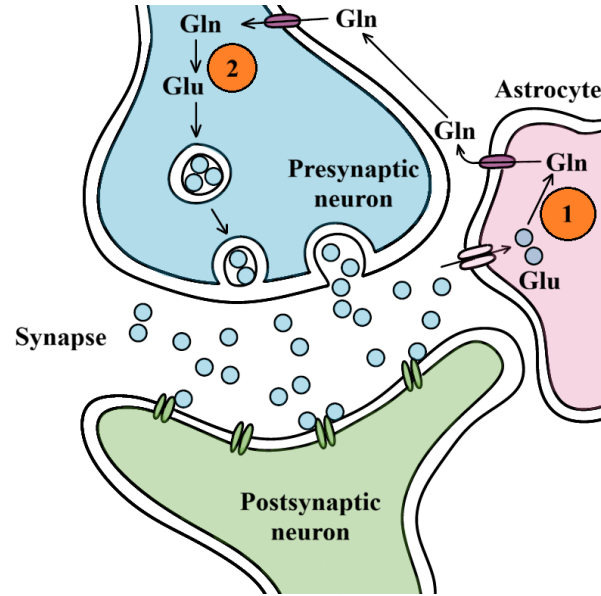


Figure 2: Schematic image of the glutamate-glutamine cycle in the human central nervous system. Glutamate is released from a presynaptic neuron into the synapse, where excess glutamate transports into an adjacent astrocyte (glial cell) where it is converted into glutamine by the enzyme glutamine synthetase (1). Glutamine in the astrocyte is transferred back into the presynaptic neuron where it is converted back into glutamate by the enzyme glutaminase (2).

Glutamate is also involved in several metabolic pathways, such as peptide and protein synthesis and as a precursor of GABA[1]. The involvement of Glu in all these pathways means that Glu concentration relate both to excitatory and metabolic activity[22].

2.2 MAGNETIC RESONANCE CONTRAST AGENTS

Magnetic resonance imaging is a well established imaging technique known for its high soft tissue contrast compared to other medical imaging techniques, as well as being non-invasive. Magnetic resonance imaging provide a large range of image contrasts, for example T1-, T2 or T2*-weighted images which utilise the difference in spin relaxation rates between tissues to generate contrast [23]. Several MRI techniques make use of physical or functional aspects other than spin relaxation rates to produce their image contrast, such as diffusion [24], perfusion [25] and fMRI [26], just to mention a few.

Besides different MRI techniques there also exist contrast agents which are compounds containing physical properties that affect image contrast. The contrast agents used in clinics are gadolinium-based (Gd^{3+}), used for enhancing contrast in T1 weighted images through a decrease of longitudinal relaxation rate in water tissue [27]. When gadolinium-based contrast agents are administered they affect the relaxation rates in tissue until physically leaving the tissue, thus would affect all subsequent scans under a certain duration. The possibility of not having to inject a contrast agent was one of the motivations behind finding alternative contrast agents.

In a study published 2000, authors Ward, Aletras and Balaban [28] introduced a new kind of contrast agent as an alternative to gadolinium-based. These contrast agents probe different biological processes than clinically used gadolinium-based contrast agents, but produce similar clinical information for certain applications. These new kinds of contrast agents were diamagnetic solutes containing molecular groups with hydrogen nuclei capable of physically exchanging with hydrogen nuclei in water. This physical exchange of hydrogen nuclei between molecular environments is referred to as chemical exchange and

the authors investigated these contrast agents using the novel MRI technique called CEST imaging. These CEST contrast agents can be classified into three different categories; diamagnetic (diaCEST), paramagnetic (paraCEST) or liposome-based (lipoCEST). Endogenous mobile macromolecules, such as glutamate, is a diamagnetic contrast agent.

In the following, hydrogen nuclei (H^1 or H^+) will for simplicity be referred to as 'protons'.

2.3 PRINCIPLE OF CHEMICAL EXCHANGE SATURATION TRANSFER IMAGING

The effects of chemical exchange in 1H MRS was introduced as early as in 1951 [29, 30] and the first example of saturation transfer through chemical exchange was demonstrated by Forsén and Hoffman in 1963 [31] as a method to determine chemical exchange rates. The study by Ward, Aletras and Balaban [28] combined these principles with investigating suitable contrast agents possessing exchangeable proton sites and demonstrated a sensitivity enhancement in the detection of certain low-concentration solutes.

The advantage of conventional MRI is the use of magnetic resonance signal originating from hydrogen atoms, which are abundant in the human body in the form of water (c. 55 M, or 110 M water protons). Water molecules are either associated to metabolites, membranes and proteins or exist as free water molecules, the latter often referred to as "bulk" water. This poses a problem when something other than water is of interest, since the spectrum is dominated by the 5000-10000 times stronger water signal compared to the signal from different metabolites [32]. Proton magnetic resonance spectroscopy provide very high sensitivity of low concentration solutes and can be used to detect and quantify numerous tissue metabolites [9]. Some disadvantages of 1H MRS application lies in the long acquisition times, necessary for sampling enough signal for sufficient SNR, as well as having poor spatial resolution compared to conventional MRI [10].

Protons in a solute can reach a saturated state when irradiated by a RF pulse corresponding to their resonance frequency, also called their Larmor frequency. This saturation can transfer to surrounding water molecules through chemical exchange, causing a decreased water signal [28]. This CEST process is illustrated in Figure 3, notably simplistic but conveying the general principle.

The exchange rate of protons between the water and solute pool is relatively fast compared to the T_1 in water, which is approximately 3-4 seconds in phantoms and roughly 1-2 seconds in gray and white matter in the brain at 7 T. This results in a saturation label that has a relatively long residence time in the water pool.

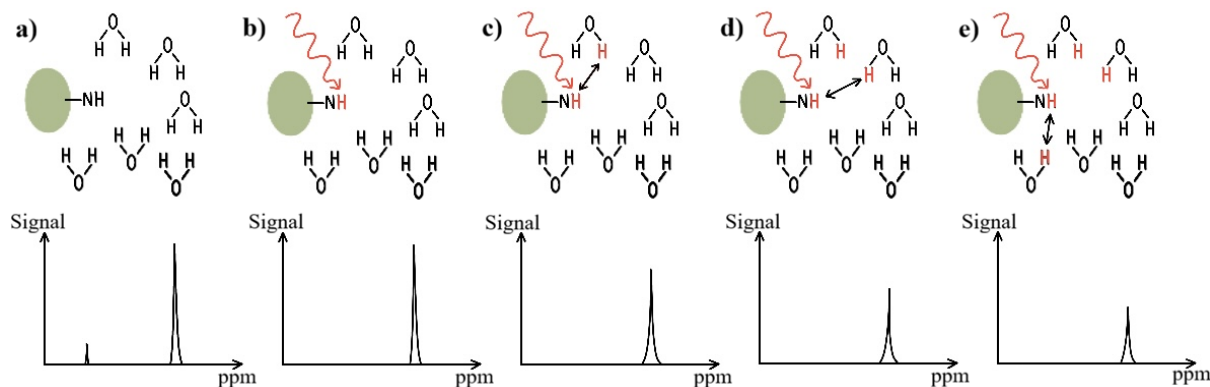


Figure 3: Illustration of the CEST process in a two-pool system, consisting of a small solute-pool with an exchangeable proton site (-NH) and a large water-pool (H₂O). At the start, a) both pools are unsaturated and no RF pulse is applied. When applied, b) the selective RF pulse saturates only the proton in the solute; c) which subsequently exchanges with an unsaturated water proton through chemical exchange, that in turn is saturated by the RF pulse. Continuous RF irradiation c) - e) allow this process to repeat; increasing the amount of saturated protons in the water pool which results in a notable loss of water signal.

What is utilised in CEST imaging is RF irradiation of protons in a solute over a relatively long period of time, on the order of seconds compared to RF pulses in conventional MRI, which are on the order of milliseconds. This long irradiation results in a cumulative saturation of the free water protons, enabling enhanced sensitivity of low concentration solutes [12]. The amplitude difference of the water signal after RF saturation (Figure 3e) compared to before (Figure 3a) is referred to as the CEST effect.

2.3.1 MAGNETISATION TRANSFER

Chemical exchange falls under the more general description of magnetisation transfer, simply denoting the transfer of longitudinal magnetisation from one molecular environment to another. The term “magnetisation transfer” however is more commonly used to describe the magnetisation transfer between semi-solid macromolecules and bulk water [33], which can sometimes confuse readers. The latter description will therefore be denoted as semi-solid magnetisation transfer (ssMT) while the general description stays as magnetisation transfer (MT).

Magnetisation transfer processes can be divided into two groups: chemical exchange and cross-relaxation through dipolar-coupling. Cross-relaxation occurs when two dipolar-coupled protons exchange magnetisation over a space [34], with a transfer efficiency inversely proportional to the distance and speed of molecular motion [35]. The intensity change due to dipolar-coupled cross-relaxation is called nuclear Overhauser enhancement (nOe) and is generally divided into different exchange pathways which result in nOe or is instigated by nOe [33]. A commonly referred nOe pathway is nOe-relayed exchange (r-nOe), where intramolecular cross-relaxation transfers magnetisation from a non-exchangeable proton in a molecule to an exchangeable proton, which transfers to water through chemical exchange. Another commonly observed pathway is intermolecular nOe, where protons in a semi-solid macromolecule get saturated and through dipolar-coupled cross-relaxation this magnetisation eventually transfers to “bound” water.

These MT processes happen simultaneously in tissue, where certain processes dominate in different molecular structures; chemical exchange for instance dominates in mobile macromolecules while intermolecular nOe dominates in semi-solid macromolecules [33].

2.3.2 THE PULSE SEQUENCE

Similar to other MRI techniques, CEST imaging is combined with different data acquisition techniques and it is common to see 2D single-shot techniques in CEST in order to shorten acquisition times [10]. The general composition of a CEST sequence consist of three parts; a preparation phase, an acquisition phase and a relaxation phase [36]. Figure 4 depict an illustration of a CEST sequence over one repetition.

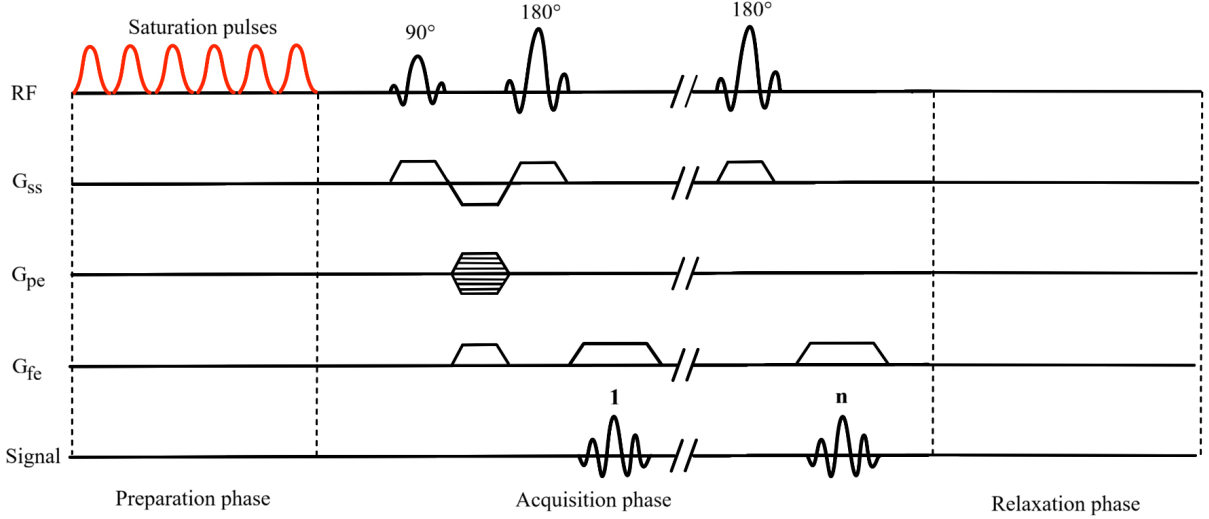


Figure 4: Pulse diagram of a CEST sequence with RF pulses, slice-selective gradient (G_{ss}), phase-encoding gradient (G_{pe}), frequency-encoding gradient (G_{fe}) and signal. A train of RF pulses make up the preparation phase followed by data acquisition (depicted as a fast spin echo (FSE) technique) with n number of sampled K-space lines. Lastly, a relaxation phase takes place before the next repetition.

The saturation phase in Figure 4 depict pulsed saturation, consisting of a RF pulse train, each pulse with defined shape, pulse duration and time between pulses (so called inter-pulse delay). These parameters allow an increased degree of freedom in optimising the CEST sequence for maximum CEST effect, but also complicate analytical comparisons and simulations [37].

Another option for RF saturation is a single rectangular saturation pulse, so called continuous wave saturation, which allow simpler analytical descriptions compared to pulsed saturation. Clinical application of continuous wave saturation is however often limited by SAR or MRI hardware, where pulsed saturation allow sufficient CEST effect with less burden on hardware or SAR [10].

Using ultra-high magnetic field strength (≥ 7 T) is advantageous since longitudinal relaxation time in bulk water ($T1_w$) increases, carrying saturation longer and thus increasing SNR. A consequence of increased $T1_w$ is a longer repetition time, since the relaxation phase ensures no saturation from previous acquisition spills over into the next by choosing $TR \gg T1_w$.

2.3.3 THE Z-SPECTRUM AND ASYMMETRIC MAGNETIC TRANSFER RATIO

The RF saturation pulse is not perfectly selective, causing it to saturate other protons than those in the targeted solute, as well as directly saturating water protons, so called direct saturation (DS). Solid and semi-solid macromolecules have a very broad spectral line, on the range of 10-50 kHz (ca. 30-170 ppm), due to their very short transversal relaxation times ($T2$) [38]. These effects complicate the CEST analysis, since they all contribute to the observed CEST effect at the resonance frequency of interest. It is therefore common in CEST imaging to acquire images at different saturation frequencies in order to analyse the

change of water signal intensity over a certain frequency interval [37]. The water signal is normalised based on the image sampled without saturation pulses (the off-resonance measurement S_0) and commonly displayed as a function of the saturation frequency $Z(\Delta\omega)$, see Equation 1 and representative behaviour in Figure 5.

$$Z(\Delta\omega) = \frac{S_{\text{sat}}(\Delta\omega)}{S_0} \quad (1)$$

Unlike ^1H MRS, the Z-spectrum display frequencies in relation to the resonance frequency of bulk water, which is 4.7 ppm but defined as 0 ppm in the Z-spectrum. The chemical shift $\Delta\omega$ is therefore referred to as a frequency offset. Traditionally the Z-spectrum is displayed with positive shift to the left of 0 ppm (called downfield) and negative shift to the right of 0 ppm (called upfield).

Generally in CEST analysis the contributing DS and ssMT effects to the Z-spectrum are assumed symmetric around 0 ppm [10]. To eliminate these effects, the asymmetric magnetisation transfer ratio (MTR_{asym}), shown in Equation 2, is commonly used as a metric of the CEST effect. The $S_{\text{sat}}(+\Delta\omega)$ and $S_{\text{sat}}(-\Delta\omega)$ is the measured water signal at frequency offset $\pm\Delta\omega$ respectively, as illustrated in Figure 5.

$$\text{MTR}_{\text{asym}}(+\Delta\omega) = \frac{S_{\text{sat}}(-\Delta\omega) - S_{\text{sat}}(+\Delta\omega)}{S_0} \quad (2)$$

It is however demonstrated that ssMT is slightly asymmetric in tissue, with an upfield minimum, and effects from r-nOe interactions are also present upfield, which affect MTR_{asym} [37]. It is therefore important to analyse the Z-spectrum when discussing CEST effects, since MTR_{asym} has contributions from different MT processes.

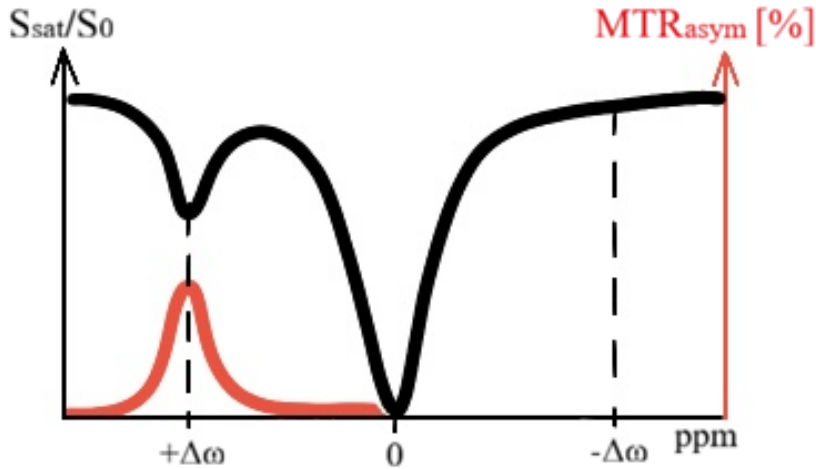


Figure 5: Illustration of Z-spectrum and corresponding asymmetric magnetisation transfer ratio (MTR_{asym}) with CEST effect at frequency $+\Delta\omega$ ppm in relation to water resonance frequency at 0 ppm.

2.4 BLOCH-McCONNELL EQUATIONS

The effect of chemical exchange on magnetisation was included in the Bloch equations in 1958 by McConnell [39], referred to as the Bloch-McConnell equations. The equations describe the dynamics between a small proton pool that transfers saturation through chemical exchange to a much larger proton pool, a so called two-pool system.

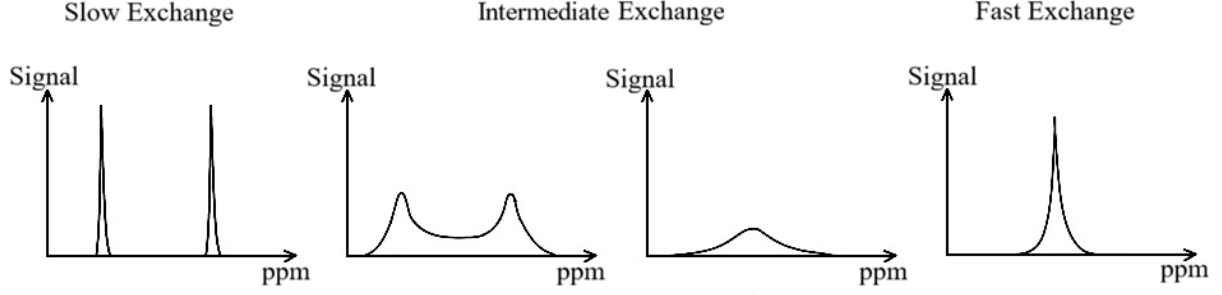


Figure 6: Illustration of spectrum from two proton pools with different resonance frequency during slow, intermediate and fast exchange between the two pools.

By defining a complex magnetisation $M = u + iv$, the Bloch-McConnell equations for two pools exchanging protons, for instance water (W) and a solute (S), can be rewritten in matrix form, as shown in Equation 3.

$$\frac{d}{dt} \begin{pmatrix} M_A \\ M_B \end{pmatrix} = -\mathbf{C} \begin{pmatrix} M_A \\ M_B \end{pmatrix} \quad (3)$$

The matrix $\mathbf{C}$ contain the chemical shift difference (Δcs) between proton sites A and B, the transversal relaxation time of the system (T_2) as well as the exchange rates (k_{sw}, k_{ws}) between the pools. The solution to the complex matrix is

$$M(t) = e^{\mathbf{C}t} \cdot M(0) \quad (4)$$

and its Fourier transform define the signal spectrum of the two-pool system. Assuming exchange rates much faster than the transversal relaxation rates (R_2) for both W and S pools [40] the real part of the complex signal spectrum is defined as follows

$$S(\omega) = \frac{\Delta cs^2 (k_{ws} + k_{sw})}{(\omega_w - \omega)^2 (\omega_s - \omega)^2 + (\frac{1}{2}(\omega_w + \omega_s) - \omega)^2 (k_{ws} + k_{sw})^2} M_0 \quad (5)$$

where ω_w and ω_s are resonance frequencies of protons in W and S respectively, and M_0 is the equilibrium magnetisation of the system [41]. For slow and lower intermediate exchange rates ($k_{sw} < \Delta cs$), the signal shape can be depicted as two Lorentzian peaks centered at ω_w and ω_s with widths k_{ws} and k_{sw} , respectively. As the exchange rate increases, the peaks are broadened, as shown in first and second image in Figure 6. For higher intermediate and fast exchange rates ($k_{sw} > \Delta cs$), the signal shape can be depicted as a single Lorentzian peak, centered at $\frac{1}{2}(\omega_w + \omega_s)$ with width $\Delta cs^2 (k_{ws} + k_{sw})^{-1}$. As the exchange rate increases, the peak width gets narrower again, as shown in third and fourth image in Figure 6.

Previously, only solutes with slow to intermediate exchange rates were visible in CEST imaging, but with the introduction of ultra-high magnetic field strength scanners it has become possible to observe faster exchange solutes since Δcs increase with B_0 [10]. This is advantageous since the CEST effect is proportional to the exchange rate, thus a fast exchange rate is preferable, as long as the conditions fulfil the following criteria in order to distinguish the CEST peak from the water signal.

$$k_{sw} < \Delta cs \quad (6)$$

The exchange rate is heavily sensitive to the pH and temperature of its environment, which by extension indicate the same for the CEST effect [28]. The exchange rate for amide and amine protons is primarily base-catalysed and therefore roughly depend on pH following the relation $k_{sw}(\text{pH}, T) \propto 10^{(\text{pH} - \text{pK}_w(T))}$,

where pK_w is the acidity constant of water in logarithmic form [42]. Therefore, CEST has since its discovery been used frequently for pH mapping and phantom measurements commonly use physiological pH and temperature in order to represent the expected CEST effect in tissue. For instance, normal brain physiological pH and temperature lie around 7 and 37°C respectively [9].

There exist several instructive publications that break down the Bloch-McConnell equations and calculate the CEST effect based on certain approximations and assumptions [12, 31, 36, 37]. One solution by Zhou and van Zijl [12] is the proton transfer ratio (PTR), assuming slow exchange rate from solute to water k_{sw} , no back exchange of the saturated protons and no DS of the water pool.

$$PTR = \frac{k_{sw} \cdot \alpha \cdot M_{0,s}}{(R1_w + k_{ws})M_{0,w}} \left(1 - e^{-(R1_w + k_{ws})T_{sat}}\right) \quad (7)$$

The PTR depend on forward exchange k_{sw} as well as back exchange k_{ws} , a saturation efficiency α dependent on B1, longitudinal relaxation rate of water $R1_w$, magnetisation of solute and water at equilibrium ($M_{0,s}$ and $M_{0,w}$) and duration of saturation pulse T_{sat} . A higher solute concentration result in higher exchangeable proton concentrations, which are proportional to the equilibrium magnetisation $M_{0,s}$ and thus a higher CEST effect. Equation 7 similarly highlight the advantages of using higher magnetic field strengths, since higher B0 decrease R1. The metric MTR_{asym} would be equal to PTR if solely caused by chemical exchange [43], but (as mentioned in section 2.3.3) MTR_{asym} is influenced by other MT processes.

The saturation efficiency α in Equation 7 is unitless and assume a value between 0 and 1. It has an on resonance ($\omega = \omega_s$) approximation for mobile solutes in steady state that follows:

$$\alpha \approx \frac{\omega_1^2}{\omega_1^2 + k_{sw}^2} \quad (8)$$

where $\omega_1 = \gamma B1$ is the nutation frequency of the saturation RF pulse [36]. CEST imaging of solutes with fast exchange rates therefore need higher saturation power (B1) to increase the saturation efficiency. However, increasing the amplitude of the saturation RF pulse while maintaining constant bandwidth result in a broadening of the peaks in the Z-spectrum, called power broadening. Both the water and solute peak can be expressed as Lorentzian functions using the Bloch-McConnell equations, with respective linewidths [36]

$$\Gamma_w = 2\sqrt{R2_w^2 + \frac{R2_w}{R1_w}\omega_1^2} \quad (9)$$

$$\Gamma_s = 2\sqrt{(R2_s + k_{sw})^2 + \frac{R2_s + k_{sw}}{k_{sw}}\omega_1^2}. \quad (10)$$

The $R1_w, R1_s$ is the longitudinal relaxation rates for water and solute respectively and $R2_w, R2_s$ is the transverse relaxation rates. The broadening of the peaks when increasing saturation power (B1) becomes clear through Equation 9 and Equation 10 when ω_1 is relatively large. For weak saturation power, the effect from B1 is negligible and the linewidth predominantly depend on relaxation rates and exchange rate.

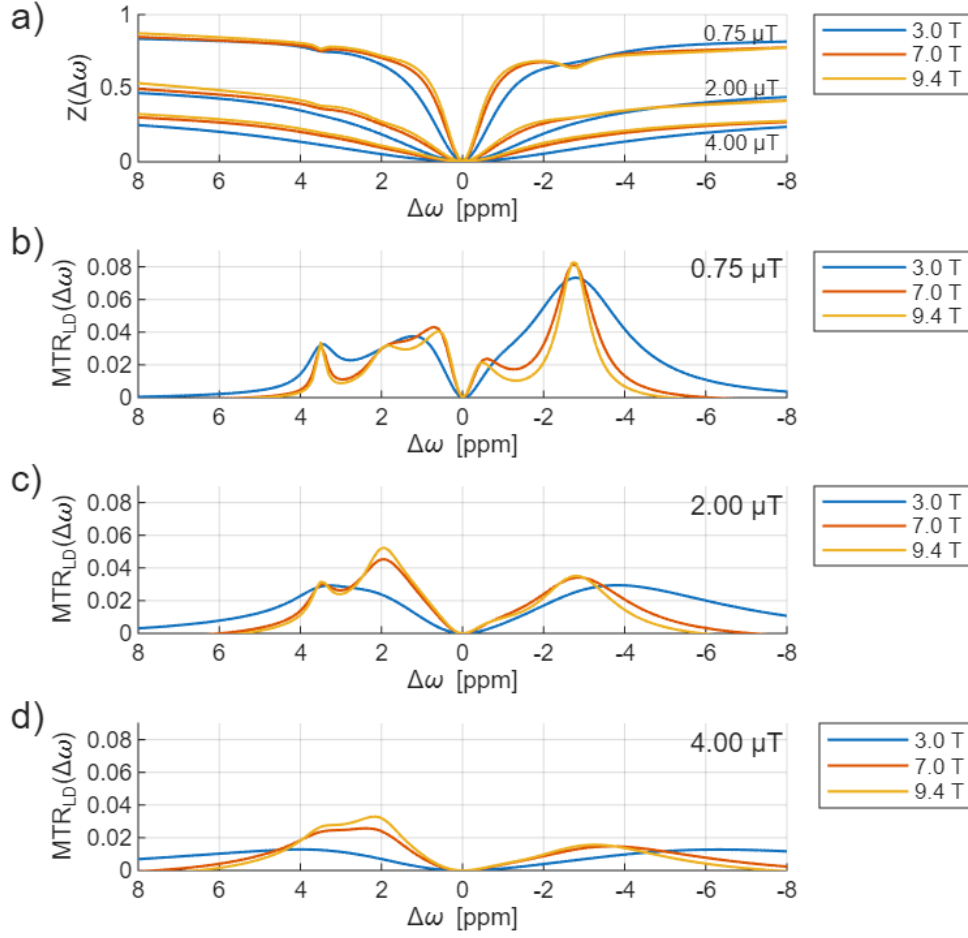


Figure 7: a) Simulated Z-spectrum and b) - d) MTR_{LD} in brain white matter, as a function of frequency offset ($\Delta\omega$) at different B_0 values and B_1 amplitudes. Simulation uses seven pools, consisting of water at 0 ppm, ssMT at -3.5 ppm, amides at 3.5 ppm, guanidine at 2 ppm, amines at 3 ppm, hydroxyl at 1.3 ppm and r-nOe at -2.75 ppm. Figures are produced using code provided by Zaiss et al. (https://github.com/cest-sources/NBM2022_CEST-B0) [36]

Another method for analysing the Z-spectrum, that does not assume symmetric ssMT or DS, instead assume a number of separate proton pools (water, solute, ssMT, r-nOe, etc.) that are assigned a Lorentzian line shape [33]. The Z-spectrum is curve fitted as the combined effect from each pool subtracted from a constant baseline Z_{base} , resulting in a labelled Z-spectral intensity Z_{lab} . The CEST effect can be separated from the background using Equation 11 to calculate the linear difference magnetisation transfer ratio (MTR_{LD})

$$MTR_{LD} = Z_{lab} - Z_{ref} \quad (11)$$

where Z_{ref} denotes the contribution from a specific pool subtracted from Z_{lab} [36].

Both MTR_{asym} and MTR_{LD} display similar changes to the Z-spectrum depending on B_0 and B_1 , shown in Figure 7 for MTR_{LD} . As visible in Figure 7, DS and spillover (overlap of water resonance and other MT resonance) decrease with B_0 and increase with B_1 , as indicated by the resonance widths. The effect from ssMT is observed as a drop of amplitude in the Z-spectrum and is shown to increase with B_1 [36].

2.5 INHOMOGENEITY CORRECTIONS

As previously mentioned, the saturation efficiency and spillover effect depend on B1 while chemical shift and longitudinal relaxation rate depend on B0. Inhomogeneities in both of these fields occur, with varying severity depending on MRI system, magnetic field strength and shimming protocols. These inhomogeneities can cause problems in CEST analysis and possibly skew results if not corrected for.

Inhomogeneities in B0 causes a position-dependent shift of the entire Z-spectrum, where the assumed frequency offsets does not align with what the phantom or tissue experience. Corrections can be done by tightly sampling close to 0 ppm using a short and weak RF saturation pulse, identifying the minimum and calculating the frequency shift for each pixel, referred to as the WASSR (WATER Saturation Shift Referencing) method [15]. Other methods consist of acquiring a separate B0 map, for instance by a dual-echo method, a fast spin-echo Dixon method [16] or a simultaneous mapping of both B0 and B1 using a WASABI (WATER Shift And B1) acquisition method [20].

Inhomogeneities in B1 result in saturation efficiency that varies spacially, possibly resulting in insufficient saturation of the protons in the solute [37]. These inhomogeneities especially affect CEST imaging of fast exchange solutes, such as glutamate. Correction for B1 are generally more time-consuming since many methods require several acquisitions over a range of B1 amplitudes in order to calculate a calibration curve [44, 45]. An alternative method using only a B1 and T1 map has been introduced by Cember et al. [46], which provides faster scan times as well as compensating for the underlying T1 dependency in the CEST contrast. For all mentioned B1 corrections there is a need for acquiring B1 maps. Alternatives for acquiring these maps include double-angle methods, signal ratios or stimulated echos, where the Dual Refocusing Echo Acquisition Mode (DREAM) method [18] is an example of the latter.

2.6 GLUTAMATE WEIGHTED CHEMICAL EXCHANGE SATURATION TRANSFER IMAGING

The amine group ($-\text{NH}_2$) in Glu, see Figure 8 is known to be an exchangeable proton site for chemical exchange with water protons and in 2012, Cai et al. [11] demonstrated that Glu was a suitable agent for CEST imaging. Cai et al. reported that Glu has a fast exchange rate of $k_{\text{sw}} \approx 5500 \pm 500 \text{ s}^{-1}$ at physiological pH, temperature and glutamate concentration found in the human brain. The amine group in Glu is generally difficult to observe in ^1H MRS because of the fast exchange rate, but results by Cai et al. showed a clear peak at 3 ppm on the Z-spectrum and MTR_{asym} for lower pH values, which moved towards ~ 1.2 ppm for higher pH values. Despite the peak shift at higher pH it is customary to keep using 3 ppm as targeted frequency offset in GluCEST, thus the MTR_{asym} increase with pH value up till $\sim \text{pH } 6$, where it then decreases with higher pH.

The ^1H MRS spectrum of Glu and Gln are very similar, only differing at the last carbon group, where Gln has an amide group ($\text{R}-\text{C}(=\text{O})-\text{NH}_2$) instead of a carboxylic acid group ($\text{R}-\text{C}(=\text{O})-\text{OH}$). Compared to ^1H MRS, these metabolites are separated more easily in CEST since the exchange rates of their amine group differ, which impact the specificity in CEST more than their distance on the ^1H MRS spectrum [14]. Khlebnikov et al. [47] investigated the contributions from different brain metabolites at 3.2 ppm in CEST and found that around 50% of the CEST effect comes from Glu, making it the dominant contributor. Since multiple metabolites contribute to the CEST effect, the most accurate term to use in CEST imaging is “weighted”, such as glutamate-weighted CEST [33].

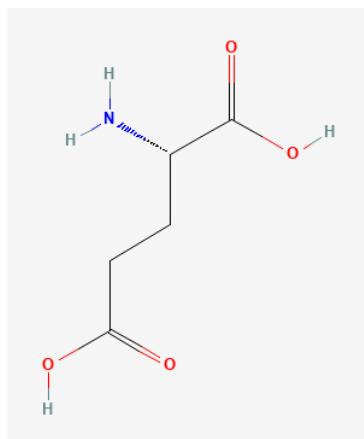


Figure 8: 2D structure of L-glutamic acid, the protonated form of glutamate. *Source: PubChem CID [33032], National Center for Biotechnology Information.* [<https://pubchem.ncbi.nlm.nih.gov/compound/L-Glutamic-Acid#section=2D-Structure>]

Since Glu has such a fast exchange rate it requires ultra-high magnetic strength of 7 T or higher to be observed in CEST imaging. At 7 T the chemical shift between the amine group in Glu and water is $\sim 5600 \text{ rad s}^{-1}$, fulfilling the criteria in Equation 6. The saturation efficiency α decreases with faster exchange rates, which require high B1 amplitudes ($> 2 \mu\text{T}$) and short saturation duration ($T_{\text{sat}} \sim 1 \text{ s}$) for sufficient CEST effect [36]. Since the CEST effect for Glu increases with B1 amplitude, a higher B1 value is preferred. Furthermore, as observed in Figure 7b, there are dominating effects upfield on the Z-spectrum at low B1 which result in negative MTR_{asym} values. The CEST effects downfield however dominate at higher B1 values, resulting in positive MTR_{asym} values, further highlighting the need for higher B1 values in glutamate-weighted CEST.

2.6.1 CURRENT APPLICATIONS

Glutamate-weighted CEST imaging has been shown to produce highly reproducible results in healthy volunteers for single-slice, pulsed CEST [13]. Results from healthy volunteers show higher MTR_{asym} in gray matter (GM) compared to white matter (WM) [11] and lower MTR_{asym} values in hippocampus of healthy old volunteers (~ 72 years) compared to healthy young volunteers (~ 22 years) [48].

Volumetric GluCEST imaging has been successfully demonstrated, with 60 1 mm slices acquired using a modified 3D FLASH technique with a segmented elliptical center encoding of the k-space [49]. This technique was used to lateralise and localise epileptogenic regions in the hippocampus of epilepsy patients, which previously had been demonstrated with single-slice GluCEST imaging [50].

The GluCEST technique has also been used to demonstrate decreased Glu concentration in the cortex and subcortex of patients on the psychosis spectrum, including Schizophrenia [51], as well as demonstrated as a possible biomarker in diffuse glioma [52] and in multiple sclerosis (MS) [53]. In preclinical studies, GluCEST has been investigated and suggested as a possible method for diagnosing and mapping early stages of Alzheimer's disease [54] and as a tool to measure glutamate deficiency in the motor cortex, correlated to Parkinson's disease [55].

An excellent in-depth article by Cember et al. [14] on GluCEST imaging, its applications as well as the advantages and limitations, is recommended for the curious reader.

3 MATERIALS AND METHODS

Initial part of the project consisted of investigating suitable CEST sequence with the help of three phantoms containing glutamate concentrations evenly distributed in a solution. Goal was to find an optimal choice of scan parameters, such as saturation pulse amplitude and pulse train duration as well as suitable number of frequency offsets and stepsize. Based on the results, a preliminary protocol was established.

The secondary part consisted of testing established protocol on healthy volunteers, followed by optimising the protocol for *in vivo* GluCEST imaging and identifying limitations as well as areas of improvement.

3.1 PROTOCOL AND PULSE SEQUENCE

All scans were performed with a Philips 7 T Achieva MRI scanner using a Nova Medical head coil (8-channel transmit/32-channel receiver array).

The protocol for CEST imaging, in order, consisted of:

- Localiser scan
- 2nd order active B0 shimming
- B1 fieldmap acquisition, DREAM sequence with 40° STEAM preparation flip angle
- B0 fieldmap acquisition, with a dual-echo sequence (added for healthy control subject (HC) scans)
- CEST sequence(s).

Philips Achieva in Lund has a sequence for CEST imaging available, designed for amide proton transfer (APT) imaging and therefore given the name APT under contrast options. This sequence was a good choice for the projects purpose since it gave access to parameter interfaces not available to the normal user. The sequence can combine with either spin echo (SE) or gradient echo (GRE) readout sequences and allow either single-slice (2D) or volumetric (3D) imaging. As a starting point, as well as to shorten acquisition times, a single axial slice (2D) was chosen for CEST imaging, without tilt or rotation and placed above the ventricles in healthy volunteer measurements.

The preparation phase consisted of pulsed saturation, with a train of 50 ms Gaussian-sine shaped pulses with no inter-pulse delay, and was followed by a single-shot FSE readout sequence. The Philips CEST sequence intended for research purposes contain customisable parameters with extended ranges as listed in Table 1.

Table 1: Parameters and ranges for the Philips CEST sequence intended for research purposes, found on the contrast page under the “APT” patch.

Parameter	Range
Frequency offset (ppm)	−10-10
Read from file	Yes/No
Z-spectrum points (ppm)	7-31 (2-128)*
Saturation B1 _{rms} (μT)	0-10
Saturation duration (s)	0-5
Delta TE (ms)	0-0.5 *
Dixon echo shifts	2-3 (0)*

* If “Read from file=Yes”, these options disappear or ranges displayed in parenthesis become available.

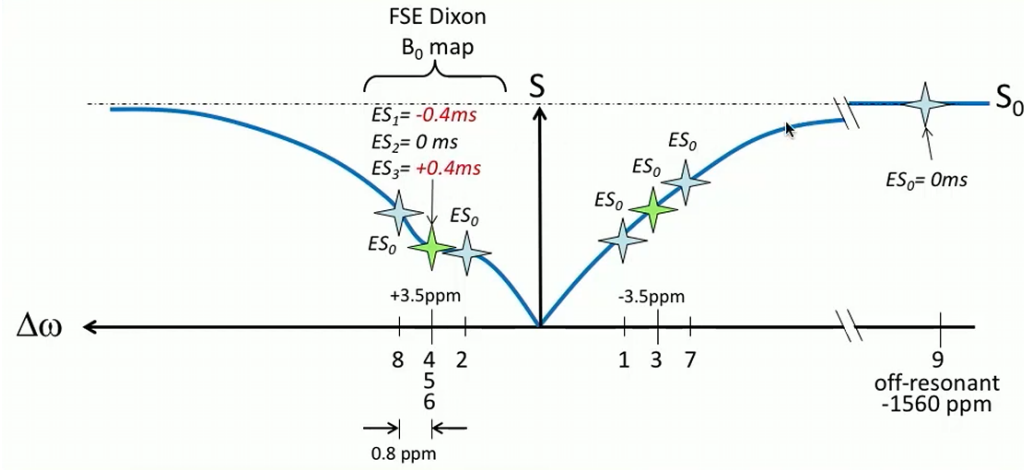


Figure 9: Example of Z-spectrum sampling scheme for seven frequency offsets using Philips CEST sequence. The order of acquisition is numbered from 1 to 9. Target in this example is APT, thus frequency offset is $\Delta\omega = 3.5$ ppm. The FSE Dixon method use the three highest downfield offsets in its acquisition in all cases except when only seven offsets are used. In that specific case, the Dixon method uses repeats scans at only one frequency offset, with a defined echo shift. Source: *Philips MRI Instruction for use : APT Extensions* [56].

By choosing “Read from file=No”, the sampling scheme shown in Figure 9 was implemented. This scheme starts with the negative frequency offset closes to water resonance and ends with the off-resonance frequency S_0 . This acquisition scheme was used for phantom scans and for the first HC scan, during which implementation using offsets from a file was under development. Using a file with frequency offsets allowed for more Z-spectrum points as well as full customisation of acquisition order and stepsize, with tighter sampling around ± 3 ppm and 0 ppm. This was preferable for a more stable interpolation and accurate B0-correction.

3.2 GLUTAMATE PHANTOM MEASUREMENT

As previously mentioned, the proton exchange rate between Glu and water k_{sw} is heavily dependant on pH, temperature and Glu concentration. The aim of the project was not to measure these dependencies. Three phantoms were used, with properties shown in Table 2, and all phantoms were scanned at room temperature, measured at 21°C.

Table 2: Phantoms with glutamate dissolved in phosphate buffered saline in water to achieve different pH-values and glutamate concentrations. K/HPO₄ is the combined volume of conjugate acid-base pair dipotassium hydrogen phosphate K₂HPO₄ and monopotassium dihydrogen phosphate KH₂PO₄.

Phantom	Volume [L]	pH	Concentration [mM]	NaCl [mM]	NiCl ₂ [mM]	K/HPO ₄ [mM]
1	1	3.12 (22°C)	147	149	0.2	-
2	0.5	4.32 (20.7°C)	20.1	150	0.2	13.8
3	0.5	7.32 (20.7°C)	20.1	150	0.2	13.8

The effect from saturation pulse amplitude (B_1) and duration (T_{sat}) on the measured MTR_{asym} was investigated. Effect from different amplitudes was investigated using $T_{sat} = 1.5$ s and repeating scans

with varying $B1_{\text{rms}}$ values (1.0, 1.7, 2.0, 2.7, 3.5, 4.0 μT). Duration was similarly investigated using $B1_{\text{rms}} = 2 \mu\text{T}$ and repeated scans with varying T_{sat} values (1.0, 1.5, 2.0, 3.0, 4.0 s).

Scan parameters for phantom measurements, with $B1_{\text{rms}} = 4 \mu\text{T}$ and $T_{\text{sat}} = 1.5$ s, are displayed in Table 3. Parallel imaging technique named SENSE (SENSitivity Encoding) [57] was used for accelerated acquisition, with a factor of 2. The three highest positive frequency offset acquisitions were used in the fast spin-echo Dixon method, with three 0.4 ms echo shifts, for B0 mapping.

3.3 HEALTHY VOLUNTEER MEASUREMENT

This project was approved by *Etikprövningsmyndigheten* (Swedish Ethical Review Authority, Dnr 2026-00579-01 P. Lundberg) and all self-reported healthy volunteers gave written consent of their participation after receiving information about the study. Three healthy volunteers (2 men, 1 woman) aged 23 to 29 years old participated in the study.

During healthy control subject (HC) scans, the addition of a separate B0 fieldmap acquisition, using a dual-echo sequence, was added. The Dixon method, acquired during the CEST sequence, was excluded after the first healthy volunteer scan.

After MR safety screening, imaging of the first participant, healthy control subject 1 (HC1), were performed with CEST acquisition parameters as detailed for phantom measurements, with saturation amplitude $B1_{\text{rms}} = 3.5 \mu\text{T}$ and duration $T_{\text{sat}} = 1.5$ s. The FOV was expanded to $200 \times 200 \text{ mm}^2$, which increased TR to 206 ms in order to stay within acceptable SAR limits. For the second and third participants (HC2 and HC3), the option “Read from file” was implemented, consisting of 67 offsets between ± 10 ppm with varying stepsize, and one off-resonance scan. The saturation amplitude $B1_{\text{rms}}$ in the CEST sequence was changed from 3.5 μT to 3.6 μT since the latter was used in other publications and would help in comparisons. The effect this change would have on the MTR_{asym} value was assumed to be negligible. Furthermore, the saturation pulse duration was changed to $T_{\text{sat}} = 1$ s to decrease the spillover effect, as well as shortening the scan time.

Imaging and CEST parameters used for phantom and HC scans are displayed in Table 3. The entire list of parameters used in the final version of the CEST sequence (used for HC2 and HC3) can be found under Appendix.

Table 3: Scan parameters for phantoms and healthy control subjects.

Parameter	Phantoms	HC1	HC2 & HC3
Geometry			
FOV (mm ²)	140x140	200x200	200x200
Pixel size (mm ²)	2x2	2x2	2x2
Slice thickness (mm)	6	6	6
SENSE-factor	2	2	2
TE/TR/shot TR (ms)	7.9/146/20298 ¹	7.9/206/16022	7.9/206/11640
Scan time (s)	629 ¹	496	792
CEST			
Frequency offset (ppm)	2.1	2.1	3
Frequency stepsize (ppm)	0.3	0.3	0.1 - 10 ²
Z-spectrum points	31	31	68
S ₀ frequency (ppm)	-1560	-1560	-600
Saturation B1 _{rms} (μT)	4 ¹	3.5	3.6
Saturation duration (s)	1.5 ¹	1.5	1
Delta TE (ms)	0.4	0.4	-
Dixon echo shift	3	3	0

¹ Depend on B1_{rms} and T_{sat}, values represent parameters used in Figure 10.

² Varying stepsize, with 0.1 ppm for closely sampled areas and up to 10 ppm further out on the Z-spectrum.

3.4 POST-PROCESSING

Image post-processing was performed with MATLAB toolbox MP³ [58] and in-house written MATLAB code for CEST analysis. The B0 fieldmap acquired with dual-echo method as well as the B1 fieldmap was realigned into the same geometry as the CEST images using the “Reslice” module in MP³, which in turn is based on the SPM12 toolbox [59].

Segmentation of phantoms and brain was performed with thresholding of the off-resonance image (S₀) and the mask was applied to all images. The CEST images were normalised with regard to S₀ and denoised using Principal Component Analysis (PCA), with a chosen amount of principal components that explained 95% of the total variance.

Normalised and denoised CEST images were corrected for B0 inhomogeneities using voxel-by-voxel pchip (Piecewise Cubic Hermite Interpolating Polynomial) interpolation of the Z-spectrum and shift of Z-spectrum based on the B0 fieldmap value. Final MTR_{asym} images were calculated from B0-corrected CEST images using Equation 2.

Fieldmaps of measured relative B1, in regard to the B1_{rms} used in the scan, was used as visual guidance in CEST analysis as well as for placement of ROIs in the MTR_{asym} images where relative B1 was 100%.

3.5 BLOCH-McCONNELL SIMULATION

Simulation of Z-spectrum in WM using varying B1_{rms} values were performed and used to compare with measurement in WM for HC2. Simulation was based on the work by Zaiss et al. [36] with their MATLAB code accessed via GitHub (https://github.com/cest-sources/NBM2022_CEST-B0).

It is based on a Bloch-McConnell system with seven exchangeable pools (water, ssMT, amide, guanidine, amine, hydroxyl, and r-nOe) and designed with a continuous wave saturation pulse. Simulation parameters are taken from *in vivo* measurements at 3 T by Wang et al. [60], Zhu et al. [61] and Stanisiz et al. [62],

with alterations to longitudinal relaxation time in ssMT ($T1_{ssMT}$) and transversal relaxation time in water ($T2_w$) for $B0 = 7$ T, shown in Table 4.

Table 4: Simulation parameters for the seven-pool Bloch-McConnell system under $B0 = 7$ T presented by Zaiss et al. [36]. Parameter f denote the exchangeable proton ratio between solute and free water.

Pool	$\Delta\omega$ (ppm)	k_{ex} (s^{-1})	f (%)	T1 (s)	T2 (s)
water	0	-	100	2.5	0.037
ssMT	-3.5	4.775	28.9	0.574	43.5e-6
amide	3.5	150	0.05	1	0.015
guanidine	2	1.1	0.09	1	0.015
amine	3	5500	0.12	1	0.015
hydroxyl	1.3	3500	0.018	1	0.015
r-nOe	-2.75	16	1.0	1	0.0025

4 RESULTS

4.1 PHANTOM MEASUREMENTS

In phantom measurements, the influence from r-nOe, ssMT or any other metabolite are not present, only DS and CEST effects. The total scan time of the entire protocol, for each phantom, was 12 minutes.

Calculated MTR_{asym} from each phantom, using Equation 2, are displayed in Figure 10 together with respective B0 and B1 fieldmaps. The three phantoms have different pH values and glutamate concentrations, thus varying MTR_{asym} values between them are expected. Within a small ROI, placed where relative $B1 = 100\%$, Phantom 1 showed the highest MTR_{asym} value at $13.2 \pm 0.2\%$, closely followed by Phantom 2 with $10.1 \pm 0.3\%$ and lastly Phantom 3 with $2.9 \pm 0.3\%$.

The gradient appearance observed in the B0 fieldmaps, shown in Figure 10b) with a range of frequency shift around ± 50 Hz (± 0.16 ppm), appear to be absent in B0-corrected MTR_{asym} images.

The MTR_{asym} values across each phantom image in Figure 10a) are not even, most clearly observed in Phantom 1 as a “half circle” of lower values, which does not correspond to the even distribution of Glu inside each phantom. Similar areas of high or low values can be observed in the respective B1 fieldmaps, see Figure 10c). The range of measured relative $B1_{\text{rms}}$ was 70-130% (2.8-5.2 μT), with nominal $B1_{\text{rms}} = 4 \mu\text{T}$.

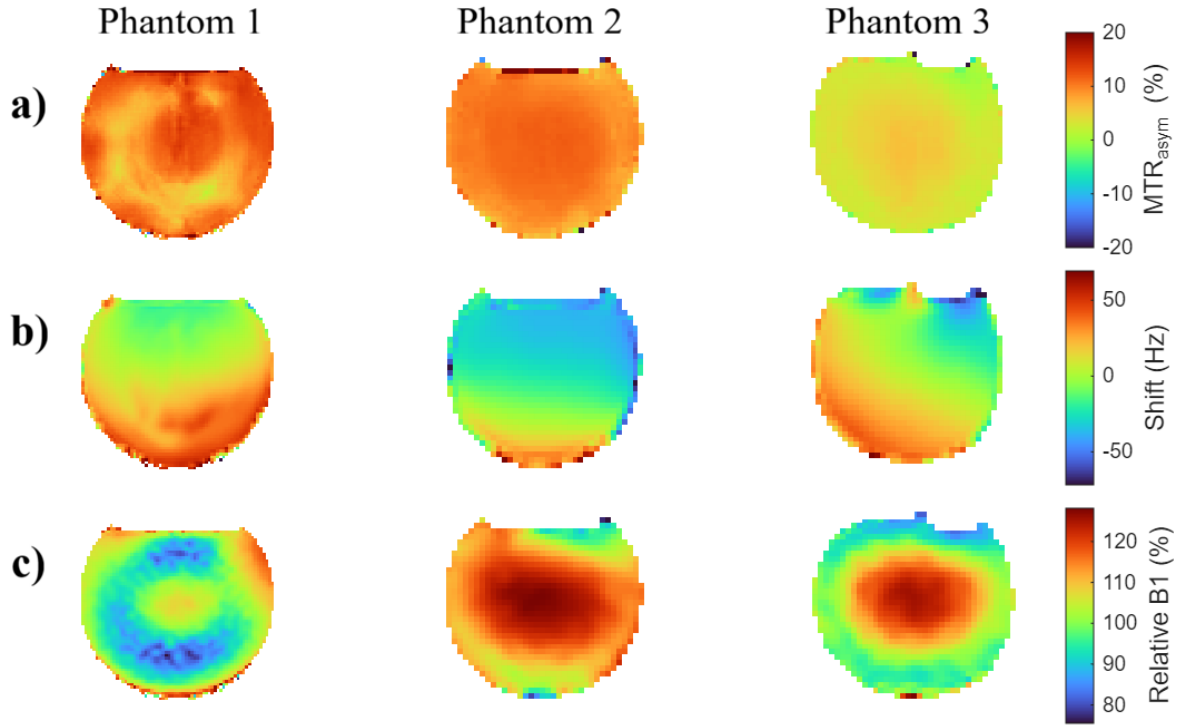


Figure 10: Scans of phantoms 1-3, using $B1_{\text{rms}} = 4 \mu\text{T}$ and $T_{\text{sat}} = 1.5$ s. a) Calculated MTR_{asym} images, which are B0-corrected based on b) B0 fieldmaps, corresponding to the same slice as a). c) Relative B1 fieldmaps, in regards to nominal $B1_{\text{rms}} = 4 \mu\text{T}$, corresponding to the same slice as a). Images of Phantoms 2 (pH 4) and 3 (pH 7) were zoomed in to match the larger size of Phantom 1 (pH 3).

4.2 RADIOFREQUENCY PULSE AMPLITUDE AND DURATION

Resulting MTR_{asym} values from scans with varying saturation RF pulse amplitudes or duration is displayed in Figure 11. For each phantom, the mean MTR_{asym} was calculated using a small ROI, placed with guidance from B1 fieldmap where mean relative $B1 = 100\%$, in order to minimise the effect from B1 inhomogeneities. The total scan time of the entire protocol was as shortest 2 minutes and 35 seconds ($B1_{\text{rms}} = 1\ \mu\text{T}$ and $T_{\text{sat}} = 1.5\ \text{s}$) and as longest 12 minutes ($B1_{\text{rms}} = 4\ \mu\text{T}$ and $T_{\text{sat}} = 1.5\ \text{s}$)

An increase in MTR_{asym} as a function of saturation pulse amplitude and duration is observed in Figure 11, except for Phantom 2 and 3 at $B1_{\text{rms}} = 4\ \mu\text{T}$. For lower saturation amplitudes ($B1_{\text{rms}} \leq 2\ \mu\text{T}$), the highest MTR_{asym} was measured in Phantom 1 while for higher $B1_{\text{rms}}$ the highest measured MTR_{asym} was found in Phantom 2.

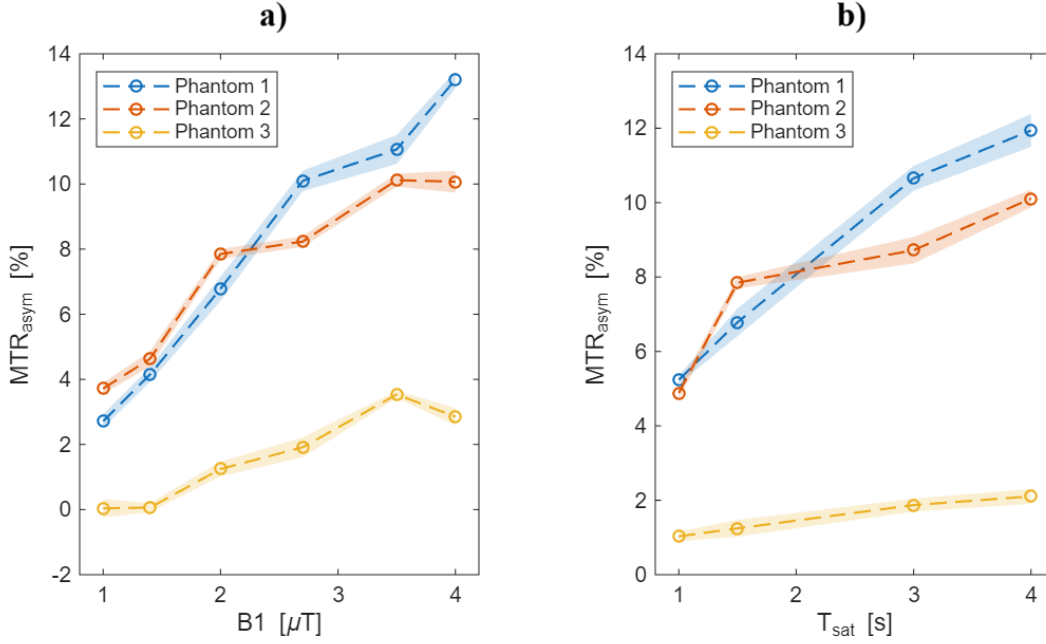


Figure 11: Measured mean MTR_{asym} and standard deviation in small ROI, placed in phantom with mean relative $B1_{\text{rms}} = 100\%$. a) MTR_{asym} as a function of saturation pulse amplitude $B1$ ($B1_{\text{rms}}$) and b) saturation pulse duration T_{sat} .

The effects that $B1_{\text{rms}}$ have on the Z-spectrum, in WM of HC2, is shown in Figure 12. The simulated Z-spectrum in Figure 12a show a positive effect on the width of Resonances 1-3 (amide, water and r-nOe) when $B1_{\text{rms}}$ increases. As well as on ssMT, observed as a decrease in normalised signal amplitude as $B1_{\text{rms}}$ increases.

Measured Z-spectrum, with same $B1_{\text{rms}}$ as simulated Z-spectra, is shown in Figure 12b. The width of Resonance 2, determined by DS, is notably slimmer for all lines in Figure 12b compared to Figure 12a, while Resonances 1 and 3 appear compressed.

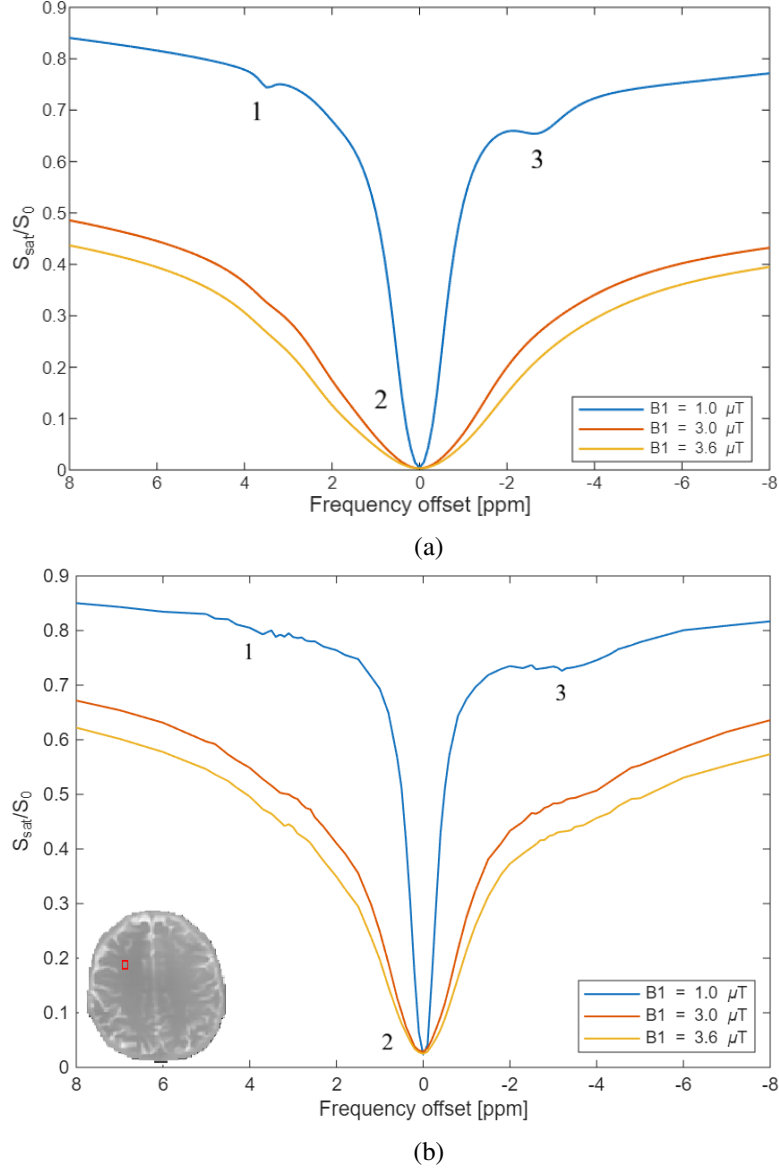


Figure 12: Z-spectrum from varying $B_{1\text{rms}}$ amplitudes with numbered resonances for 1) amide at 3.5 ppm, 2) water at 0 ppm and 3) r-nOe at -3.5 ppm. a) Z-spectrum simulated using adapted code from the seven-pool Bloch-McConnell system presented by Zaiss et al. [36], with values from Table 4. b) Averaged Z-spectrum in WM of HC2, measured in a (red) square ROI (5x5 pixels) with mean relative $B_1 = 100\%$, using $T_{\text{sat}} = 1$ s.

4.3 HEALTHY CONTROL MEASUREMENTS

Healthy control subjects were scanned with parameters shown in Table 3, one set for HC1 and another for HC2 and HC3. The B_0 -corrected MTR_{asym} images, acquired relative B_1 maps and T1w image for each healthy volunteer is displayed in Figure 13. The total scan time of the entire protocol was 9 minutes and 48 seconds for HC1, and 14 minutes and 43 seconds for HC2 and HC3.

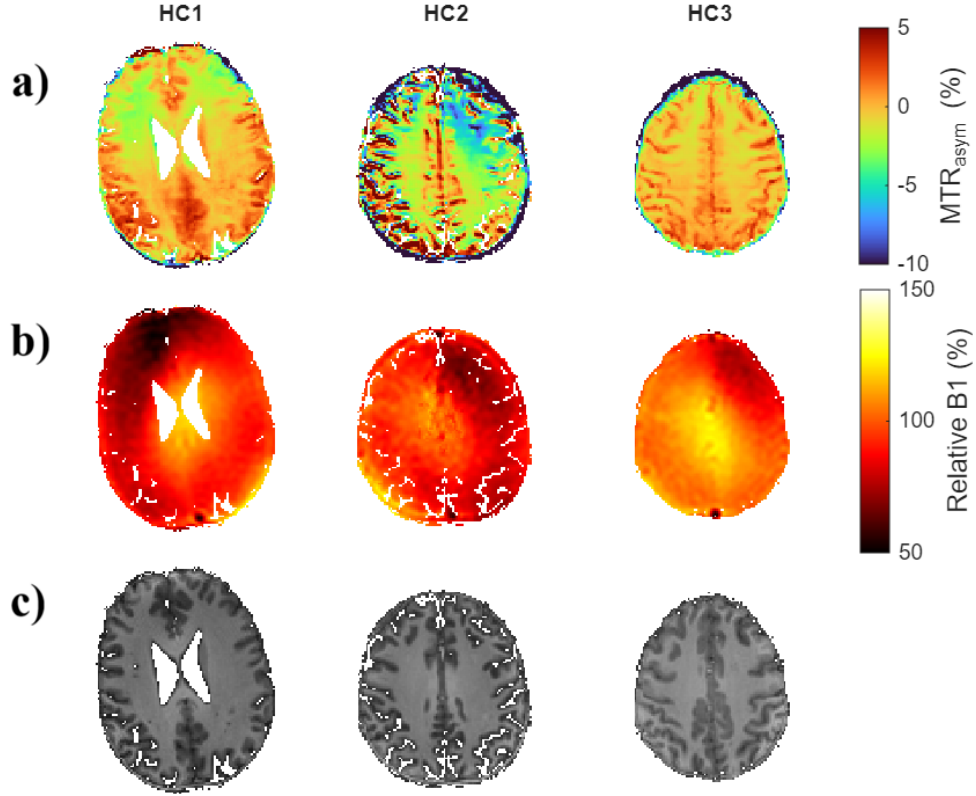


Figure 13: Glutamate-weighted CEST imaging of HC with a) B0-corrected MTR_{asym} images at 3 ppm, b) relative B1 maps of same slice as a), as well as c) T1w anatomical images from localiser scan of the same slice as a).

Guided by the relative B1 maps in Figure 13b) and with the help of the T1-weighted images in Figure 13c) because of superior tissue contrast, one ROI was drawn in WM and three smaller ROIs in GM. Three ROIs were drawn in GM in order to reach similar ROI area as the one in WM. Each ROI had mean relative $B1_{rms} = 100\%$ and the measured MTR_{asym} in WM and GM is presented in Table 5. Measured MTR_{asym} in WM for all volunteers was negative, caused by a lower upfield signal, as shown in Figure 15 where the r-nOe resonance at -3.5 ppm dips lower than the Glu resonance at +3 ppm.

Table 5: Measured MTR_{asym} in healthy volunteers, mean value and standard deviation, in a small ROI placed in WM and three small ROIs placed in GM.

Healthy control subject	WM [%]	GM [%]
HC1	-0.30 ± 0.18	1.89 ± 0.85
HC2	-1.02 ± 0.46	1.0 ± 2.6
HC3	-0.59 ± 0.45	0.35 ± 0.94

Notable in scans of HC1 in Figure 13 is the inclusion of the ventricles in each scan. A test-retest was performed on HC1 and the slice position in relation to the scanner was unchanged after participant was repositioned, thus a different anatomical slice was scanned. The reason images from after reposition was used stems from observations in Figure 14 and Figure 15. Starting with Figure 14 (B0 maps acquired from two different methods) there was a notable difference in measured frequency shifts between the dual-echo method, Figure 14a), and the Dixon method, Figure 14b). The dual-echo B0 map was acquired after the reposition of HC1 and could only be realigned to the geometry of the scan from the retest.

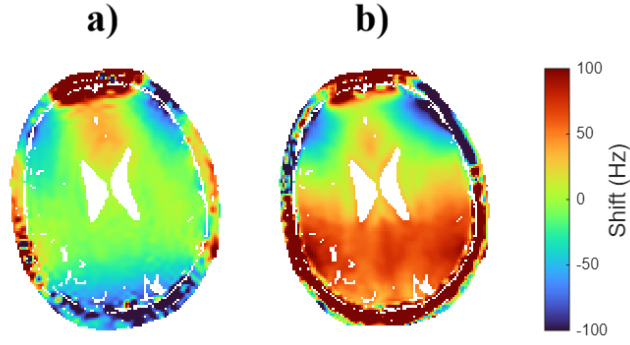


Figure 14: Acquired B0 maps of HC1 with a) separate B0 map acquisition using a dual-echo method and b) a CEST sequence integrated 3-point Dixon method.

Continuing with Figure 15 (Z-spectrum in WM of HC1) a suspiciously similar signal was observed from the three highest downfield offsets. These are the corresponding offsets used for the 3-point Dixon method in order to acquire B0 maps, displayed in Figure 14b). For reasons explained in the Discussion, the dual-echo map was used for B0-correction for HC1 and added to the CEST protocol for all subsequent measurements, while the Dixon method was excluded from the CEST sequence.

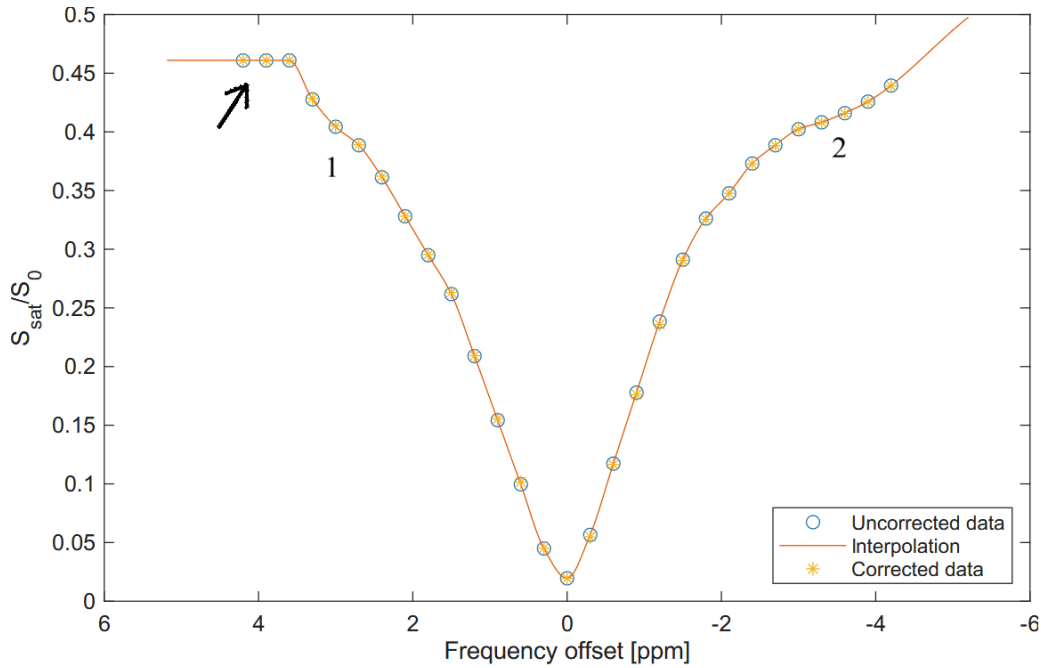


Figure 15: Z-spectrum in WM of HC1 with 30 evenly spaced offsets between ± 4.2 ppm and numbered resonance 1) show CEST resonance from Glu at 3 ppm and 2) r-nOe resonance at -3.5 ppm. Uncorrected data show measured signal after normalisation but before B0-correction. Orange line show pchip interpolation of uncorrected data points and orange stars show B0-corrected data points. Indicated by an arrow are the three frequency offsets used in the 3-point Dixon acquisition, with the same relative intensity.

5 DISCUSSION

5.1 PHANTOM MEASUREMENTS

Measured MTR_{asym} in phantoms correspond well to results by Cai et al. [11], however a slight difference in values was expected since the authors normalise their Z-spectrum based on the $S_{\text{sat}}(-3 \text{ ppm})$ scan, instead of using the off-resonance scan S_0 . If the only difference between Phantoms 1-3 was pH, a higher MTR_{asym} at 3 ppm would be expected for Phantom 2 instead of Phantom 1. Lower pH decrease the exchange rate k_{ex} which from Equation 7 is shown to be proportional to the CEST effect. The reason why Phantom 1 has stronger CEST effect despite this, is the almost 7.5 times larger glutamate concentration compared to Phantoms 2 and 3. The PTR in Equation 7 increase with glutamate concentration as well as pH, with a linear relation for lower concentrations (0-10 mM) as demonstrated by Cai et al. For concentrations as large as 147 mM in Phantom 1 this linearity seemingly breaks down, possibly explained by an increased amount of back exchange. How the CEST effect, or more specifically MTR_{asym} scales at high glutamate concentration has not been demonstrated and would need to be further investigated. The exchange rate k_{ex} roughly decrease by 20% per 0.1 pH decrease and roughly 7% per 1 °C. In phantom measurements by Cai et al. [11], the MTR_{asym} at pH 3, 4, and 7 was roughly 12, 13 and 4% respectively ($B1_{\text{rms}}=3.6 \mu\text{T}$, $T_{\text{sat}}=1 \text{ s}$), indicating other more dominating effect on the MTR_{asym} value than only pH value.

As discussed further in section 5.3, the truncating effect on scans with frequency offsets used for the Dixon method, shown in Figure 15, was observed clearly in the first healthy control subject. Retrospectively, an almost identical signal was measured from the three most downfield offsets, but since their corresponding Z-spectrum was nearly flat at either end of the spectrum, this was not observed in the phantoms. The frequency shift in the Dixon B0 maps is comparatively small and B0-correction shifted the Z-spectrum in correct direction and was seemingly close to 0 ppm. However, based on this, MTR_{asym} values in Figure 10a) and Figure 11 might have been affected, making interpretation of the results slightly more challenging and should be taken with some reservation. Repeated scans using dual-echo acquisition for B0 maps would likely improve the data.

A strong influence from B1 inhomogeneities were as expected observed in Figure 10a. B1 inhomogeneities are stronger in 7 T MRI scanners compared to lower field strengths due to the shorter RF wavelength ($\sim 12 \text{ cm}$ in water), which results in interference patterns in materials with lengths around the same size as the wavelength. The interference patterns leads to localised lower $B1_{\text{rms}}$ values at destructive interference and localised higher $B1_{\text{rms}}$ values at constructive interference, thus the tissue does not get evenly saturated by the saturation RF pulse. The reason Phantom 1 exhibit a stronger effect from B1 inhomogeneities is its larger size, being roughly twice as large. These inhomogeneities could be mitigated by using lower magnetic field strength scanners, such as 3 T. However, the chemical shift difference between glutamate and water depend on the magnetic field strength and decreases at lower field strengths. Using the estimated exchange rate in phantoms ($\sim 5500 \text{ s}^{-1}$), the value is only slightly lower compared to the chemical shift difference between glutamate and water at 7 T ($\sim 5600 \text{ rad s}^{-1}$). In order to distinguish the CEST peak from the water peak, a magnetic field strength of 7 T is needed and rather suggest that even higher field strengths are advantageous.

Placement of frequency offsets when not reading them from a file proved to be challenging and restricted to a fixed stepsize, which was not ideal. The placement of points in the Z-spectrum were automatically placed symmetrically around the target frequency offset and approached 0 ppm (of the Z-spectrum) from both sides; this meant that that an offset equal to 0 ppm would have to be sampled twice. This could be particularly useful in cases of very low SNR since the water signal is extremely weak that close to its intrinsic resonance frequency. However, sufficient signal was collected from a single acquisition for interpolating the Z-spectrum curve because of B0 inhomogeneities. The signal sampled twice was most likely close to, but not exactly, 0 ppm. Furthermore, the fixed stepsize between frequency offsets made

it impossible to both achieve sufficiently small steps while also reaching 0 ppm, as well as reaching sufficiently far out on the Z-spectrum, both upfield and downfield.

These results indicate that the CEST sequence and post-processing pipeline worked, since the estimated MTR_{asym} for each phantom correlate to previous studies. The option to use “Read from file” is preferable for more optimised sampling as well as interpolation of the Z-spectrum, and using dual-echo B0 maps was preferred. The MTR_{asym} image was strongly affected by B1 inhomogeneities and would benefit from an optimised B1-correction.

5.2 RADIOFREQUENCY PULSE AMPLITUDE AND DURATION

The choice of saturation pulse amplitude and duration vary widely between GluCEST publications, commonly using $\sim 3.5 \mu\text{T}$ for $B1_{\text{rms}}$ and $T_{\text{sat}} \sim 1$ s. To allow comparison of our measurements with other publications, these values for $B1_{\text{rms}}$ and T_{sat} were chosen. As shown in Figure 6d, the reason for higher $B1_{\text{rms}}$ comes from the dominating CEST effect downfield compared to upfield. These allow for positive MTR_{asym} is not equal to the proton transfer ratio but depends on it. Based on Equation 7, the MTR_{asym} value was therefore expected to increase with both saturation pulse amplitude and duration, which is shown in Figure 11. An increased saturation pulse amplitude lead to increased saturation efficiency α , which is favourable since a larger proton transfer ratio lead to stronger signal enhancement, thus increasing the sensitivity to detect glutamate concentration. The consequence of high $B1_{\text{rms}}$ is an increased SAR, resulting in tissue heating, which can limit the maximum available $B1_{\text{rms}}$ on the scanner. Alternatively, lengthening the repetition time as a compensation may lead to excessively long scan times. Furthermore, with too high $B1_{\text{rms}}$ values the assumption of symmetric semi-solid magnetisation transfer no longer holds as well as increasing both direct saturation and the spillover effect, resulting in broader resonances in the Z-spectrum, that also then overlap. While a higher B1 result in higher saturation efficiency α , the water and CEST peak become broader due to power broadening, as shown by Equation 9 and Equation 10. This also contributes to the spillover effect. The observed decline in MTR_{asym} value for Phantoms 2 and 3, at $4 \mu\text{T}$, can be explained by an increased spillover effect, where the overlap between Glu resonance and water resonance resulted in a smaller resonance at 3 ppm. Especially Phantom 3, which has physiological pH 7.32 and thus a faster exchange rate than Phantom 1 and 2, will have a shifted resonance closer to water and it was therefore more affected by DS as well as the spillover effect.

A positive trend was observed in Figure 11b, for $B1_{\text{rms}} = 2 \mu\text{T}$ and the MTR_{asym} value for Phantom 3 increased by $\sim 1\%$ when saturation pulse duration increased from 1 second to 4 seconds. Shorter saturation pulse duration did reduce spillover effects, direct saturation and semi-solid magnetisation transfer slightly. For that reason, a shorter T_{sat} is often adopted to mitigate the effects caused by large $B1_{\text{rms}}$ values; but it should not be too short as that would lead to insufficient saturation efficiency. It is therefore a matter of optimising the CEST-protocol depending on which molecular species that is the target. Phantom 3 had a physiological pH and therefore represented the expected CEST effect in healthy tissue, and the resulting MTR_{asym} that would be detected. The reason why no apparent decline in MTR_{asym} was observed for longer saturation pulses can be explained by the use of a relatively low $B1_{\text{rms}} = 2 \mu\text{T}$. The amount of spillover or direct saturation was not large enough for the chosen $B1_{\text{rms}}$, thus would therefore not affect the MTR_{asym} in an observable way in combination with the chosen lengths of T_{sat} used in these measurements.

Based on the results shown in Figure 11 and considering the additional effects present in tissue, a saturation pulse amplitude of $3.5 \mu\text{T}$ and total pulse train duration of 1.5 seconds was chosen for the scan of the first healthy control subject.

Variations in the relative $B1_{\text{rms}}$ map was observed and exploration of how the measured Z-spectrum depended on different $B1_{\text{rms}}$ values was motivated. Using a code adapted from Figure 7a), the

Z-spectrum at 1 μT , 3 μT and 3.6 μT using a continuous wave saturation pulse was simulated. The width of Resonances 2 was smaller for all lines in Figure 12b compared to Figure 12a. The difference could be explained by the use of a continuous wave pulse in the code compared to pulsed saturation in Figure 12b. The effective saturation pulse amplitude for the continuous wave in the simulation was therefore not equivalent to the $B1_{\text{rms}}$ of the pulse train used in measurements. Together with that, the continuous wave simulation assumes that the signal reaches steady state, which often is not the case with pulsed saturation. Thus, the simulation probably overestimates magnetisation transfer and spillover effects, which would explain the observed difference between measured and simulated Z-spectrum in Figure 12. Furthermore, the values of $T1_{\text{ssMT}}$ and $T2_w$ used to migrate the simulation from 3 T to 7 T were possibly not sufficiently accurate and as the simulation was altered slightly to match *in vivo* data there might exist a bias. Yet another factor is that the assumed exchange rate in the simulation for the anime group (5500 s^{-1}) may not agree with the actual exchange rates in white matter. Since the glutamate exchange rate at physiological pH and temperature was estimated in phantoms, the actual exchange rate *in vivo* might not be the same. However, the only reported exchange rate of glutamate that was found has been measured in rat brains ($\sim 2500 \text{ s}^{-1}$). While the true exchange rate of glutamate could possibly be somewhere between these values, there would still be a need for magnetic field strength of 7 T to observe the CEST peak at glutamate resonance (3 ppm). The chemical shift of glutamate at 3 T is $\sim 2400 \text{ rad s}^{-1}$ which would be too low.

The water resonance, Resonance 2, did not completely reach zero at 0 ppm for any line in Figure 12b, as opposed to what was expected and observed in Figure 12a. Among other things, this was caused by the always present thermal noise in MRI, called the noise floor, and consist of random signals generated by the electronics and the scanned item or person. The signal acquired in magnetic resonance contain both real and imaginary components, where the complex data is therefore typically converted into magnitude images. This is done by taking the absolute value of the acquired signal, which added up the noise present in both component, making it non-zero (and positive).

It was apparent in both Figure 12a and Figure 12b that $B1_{\text{rms}}$ inhomogeneity can strongly affect the Z-spectra. $B1_{\text{rms}}$ variations up to $\pm 50\%$, as measured in Figure 13b), lead to areas that experienced very different saturation efficiency which was something that affected the calculated MTR_{asym} . $B1$ -correction is therefore strongly recommended in post-process for more accurate CEST imaging. This could possibly be achieved by using the Olea Medical software for CEST analysis, with both $B0$ - and $B1$ -correction. These post-process $B1$ corrections could maybe be combined with methods that reduce $B1$ inhomogeneities during CEST imaging. For instance, with the use of dielectric pads which Jacobs et al. [63] has shown to decrease $B1$ inhomogeneities across the image, or with the use of active $B1$ -shimming methods [64].

5.3 HEALTHY CONTROL MEASUREMENTS

As shown in Figure 12, Figure 13 and Table 5, the upfield signal at -3 ppm dips lower than the downfield signal at +3 ppm, resulting in negative MTR_{asym} . As mentioned in Theory, MTR_{asym} is affected by more than the CEST effect from the solute of interest. Most notable are the effects from direct saturation, semi-solid magnetisation transfer and nuclear Overhauser enhancement relayed exchange. Since the exchange rates at physiological pH causes the CEST peak to shift towards the water peak, the MTR_{asym} value decreases at 3 ppm. At the same time, magnetisation transfer effects from r-nuclear Overhauser enhancement relayed exchange and semi-solid magnetisation transfer are now present in the Z-spectrum of tissues, as compared to phantom measurements. The asymmetry of semi-solid magnetisation transfer became more apparent in higher $B1_{\text{rms}}$, dominating upfield, which causes a decrease in signal more at -3 ppm than at +3 ppm. Similarly, the presence of nuclear Overhauser enhancement relayed exchange in tissue show as a clear resonance at -3.5 ppm, which further causes a dominating saturation effect at -3 ppm, resulting in negative MTR_{asym} values. Therefore, a negative value does not reflect an absent CEST

effect from Glu, only that upfield effects dominate. Analysing the Z-spectrum thus becomes important, possibly most suitably using n-pool Lorentzian curve fitting and using the MTR_{LD} metric, requiring evenly distributed offsets for well characterised semi-solid magnetisation transfer and direct saturation. In order to try and lower the effects from nuclear Overhauser enhancement relayed exchange, semi-solid magnetisation transfer as well as spillover effects observed in the first healthy control subject, T_{sat} was shortened to 1 second. With the idea of more easily comparing results with other publications, $B1_{rms}$ was changes from $3.5 \mu T$ to $3.6 \mu T$.

A notable contrast between white matter and gray matter (for first and second healthy control subject a $\sim 2\%$ difference and for the third subject $\sim 1\%$) was observed in Figure 13a) and Table 5. Consistent mean MTR_{asym} values in both tissue types, with notable standard deviations, but no overlap, except for in gray matter of the second healthy control subject. With measured standard deviation of 2.6%, the values in gray matter varied largely for the second healthy control subject, which could be caused by inaccurate placement of ROIs. Specifically for the second healthy control subject, there was some difficulty in identifying gray matter using the localiser images, and the standard deviation would indicate that the ROI placement could be inaccurate in certain areas. Selecting slice position with optimal separation of gray matter and white matter would be recommended. Adding a T1-weighted sequence with better contrast between gray matter and white matter would also be suggested, for instance MP2RAGE, which also could be included in B1-correction using the method demonstrated by Cember et al. [46].

The B0 map produced by the CEST sequence using a 3-point Dixon method displayed a different appearance for the first healthy control subject compared to B0 maps acquired using a dual-echo method, as is shown in Figure 14. The appearance and measured shift in the dual-echo B0 map was more consistent with reported B0 maps in other CEST publications using similar set-up [65]. Furthermore, the effect from the Dixon method on the offsets it uses for their echo shifts, as shown in Figure 15, was a further reason to replace it in the CEST protocol with the dual-echo method. In future work, implementing a WASSR method, most commonly used in previous work, would possibly produce more accurate B0 maps and can be acquired using current CEST sequence, but with much lower $B1_{rms}$. Alternatively, implementing the WASABI method would simultaneously produce B0 and B1 maps, thereby reducing the total scan time of the CEST protocol.

Similarly to phantom measurements, the influence from B1 inhomogeneities was clearly observable in the MTR_{asym} images (shown in Figure 13a). Areas with lower values corresponded to areas in the relative B1 maps that had lower values and similarly for high value areas. This further confirm the notion of implementing a B1-correction method in order to more accurately perform GluCEST imaging.

5.4 LIMITATIONS

Challenges in most types of CEST imaging are the sensitivity to tissue movement, which are not a major difficulty in brain MRI. Other challenges include B0 and B1 inhomogeneities, the latter is especially problematic in 7 T scanners. Moreover for GluCEST, the fast exchange rate make it challenging to analyse the Z-spectrum, even when using n-pool Lorentzian fitting, since there is no large amplitude glutamate resonance on the spectrum at physiological pH and temperature. Special attention to curve fitting therefore requires close sampling over a relatively large frequency interval, requiring many frequency offsets which increase the scan time.

The current scan time of 14 minutes and 43 seconds for the entire protocol (used for the second and third healthy control subject) is not unreasonably long, on its own. Motivation for shorter scan times could however arise in future implementation if these scans become an addition to an existing list of diagnostically necessary scans. Furthermore, shorter scan times are always a positive for patient comfort and thus should be investigated. For these cases, there is a wish to shorten the acquisition while maintaining the saturation phase. However, the length of read-out repetition time is limited by SAR and the total time of the CEST sequence depend on the number of frequency offsets.

6 CONCLUSIONS

In this project, detection of glutamate using GluCEST was successful in both phantom and healthy control measurements using a 7 T MRI system. This work has been exploratory, identifying possibilities and limitations to GluCEST at the Philips Achieva 7 T scanner, and further investigation is recommended. A pipeline for post-processing and CEST analysis has been implemented and can be used for further work in optimising the GluCEST protocol. Alternatively, using Olea Medicals CEST analysis pipeline could be an alternative in future clinical research projects GluCEST work.

While a reliable method for B0-correction was successfully implemented, there is incentive in exploring different methods for acquiring B0 maps, most notably using the WASSR method. Furthermore, methods for robust B1-correction is strongly recommended, where implementation of WASABI sequence for simultaneously acquisition of B0 and B1 maps could be advantageous.

Further testing of volumetric GluCEST imaging is suggested, where previous publications have demonstrated its possibility. Some articles concerning GluCEST also utilise gradient-echo readout for its generally faster acquisition times and lower SAR, thus investigation of GluCEST images acquired from gradient-echo readout is recommended.

Scans of healthy volunteers is suggested to continue in order to build a larger cohort of control subjects and the GluCEST protocol could be utilised in studying glutamate dysregulation in neurological diseases, such as Parkinson's disease, Essential Tremor, or epilepsy, which would also greatly benefit from volumetric GluCEST images. Applications on brain tumours could also be of value.

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APPENDIX

--- List of 2D TSE CEST sequence parameters ---

```
EX_single_scan_id = 42860902;
EX_ACQ_patient_weight = 58;
Nucleus = "1H";
SmartSelect = "no";
clinical modes = "NOVA40";
Coil 1 = "NOVA HEAD8 ANT RX16";
    Channels = (16) "yes",
               (16) "no";
    connection = "ODU_DCC0";
Coil 2 = "NOVA HEAD8 POST RX16";
    Channels = (16) "yes",
               (16) "no";
    connection = "ODU_DCC1";
Coil 3 = "None";
Uniformity = "CLEAR";
FOV      AP (mm) = 200;
         RL (mm) = 200;
ACQ voxel size  AP (mm) = 2;
                RL (mm) = 2;
Slice thickness (mm) = 6;
Recon voxel size AP (mm) = 1.5625;
                RL (mm) = 1.5625;
Fold-over suppression = "no";
Reconstruction matrix = 128;
SENSE = "yes";
    P reduction (RL) = 2;
CS-SENSE = "no";
k-t Acceleration = "no";
Slice orientation = "transverse";
Fold-over direction = "RL";
Fat shift direction = "P";
O-MAR = "no";
Large table movement = "no";
PlanAlign = "no";
REST slabs = 0;
    orientation = "transverse";
Interactive positioning = "no";
Patient position = "head first";
Patient body position = "head first";
Patient orientation = "supine";
Patient body orientation = "supine";
Scan type = "Imaging";
Scan mode = "2D";
    technique = "SE";
Modified SE = "no";
Acquisition mode = "cartesian";
Fast Imaging mode = "TSE";
    shot mode = "single-shot";
    startup echoes = 1;
    profile order = "low_high";
DRIVE = "yes";
```

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    ultrashort = "yes";
    fid reduction = "default";
Echoes = 1;
    partial echo = "no";
TE = "shortest";
Flip angle (deg) = 90;
Refocusing control = "constant";
    angle (deg) = 80;
    bright fat reduction = "no";
TR = "shortest";
Halfscan = "no";
Water-fat shift = "maximum";
RF Shims = "fixed";
Shim = "shimtool";
    Get shim and F0 from PRIDE = "auto";
    Calculate shim now = "no";
    F0 Determination = "auto";
X    shim value = -0.0020873379;
Y    shim value = -0.000668091583;
Z    shim value = 0.127764151;
Z2   shim value = 0.786912143;
ZX   shim value = 0.290200531;
ZY   shim value = -0.00521845929;
X2-Y2 shim value = -0.109052882;
2XY  shim value = -0.0027601216;
Z3   shim value = -0;
Z2X  shim value = -0;
Z2Y  shim value = -0;
Z(X2-Y2) shim value = -0;
2XYZ  shim value = -0;
X3   shim value = -0;
Y3   shim value = -0;
Shim offset freq = 0;
Nav shim offset freq = 0;
Navigator X shim value = 0;
Navigator Y shim value = 0;
Navigator Z shim value = 0;
ShimAlign = "no";
mDIXON = "no";
Fat suppression = "no";
Grad Rev Fat suppr = "no";
Water suppression = "no";
BB pulse = "no";
MTC = "no";
APT = "yes";
    Expert mode = "yes";
    freq offset (ppm) = 3;
    Read from file = "yes";
    Z-spectrum points = 68;
    RF pulses = 0;
    Alternate rf amp = "yes";
    Saturation B1 rms (uT) = 3.5999999;
    Saturation duration (s) = 1;
    Post sat delay = 0;

```

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    Dixon echo shifts =          0;
Custom prepulse =              "no";
MDME =                        "no";
Diffusion mode =              "no";
T1 mapping =                  "no";
SAR mode =                    "high";
B1 mode =                     "default";
SAR allow first level =       "yes";
    Patient pregnancy =        "no";
    Patient WB SAR [W/kg] =     0;
    Patient Head SAR [W/kg] =   0;
    Patient max. dB/dt [T/s] =  0;
    Max slewrate [T/m/s] =     0;
    Max. B1+rms [uT] =         0;
PNS mode =                    "moderate";
Gradient mode =               "full control";
    gradient switch =          "standard";
    max strength (mT/m) =      33;
    max slew rate (T/m/s) =    160;
PTX pulse def. id =          0;
Cardiac synchronization =     "no";
Heart rate > 250 bpm =        "no";
Respiratory compensation =    "no";
Navigator respiratory comp =  "no";
Flow compensation =           "no";
Motion smoothing =            "no";
NSA =                         1;
Manual start =                "no";
Dynamic study =               "no";
Arterial Spin labeling =      "no";
Preparation phases =          "auto";
Interactive F0 =              "no";
Pre scan =                    "no";
Quick Survey =                "default";
SmartPlan survey =            "no";
B0 field map =                "no";
B1 field map =                "no";
MIP/MPR =                     "no";
APT processing type =         "no";
APT image type =              (4) "no";
APT min SNR S0 =              21;
APT display range =           20;
Reference tissue =            "Grey matter";
Recon compression =           "No";
Preset window contrast =      "soft";
Reconstruction mode =         "real time";
Save raw data =               "yes";
Hardcopy protocol =           "no";
Image filter =                 "system default";
Uniformity correction =        "yes";
Geometry correction =          "default";
+fixed drive scale =           "no";
+Enable PO warnings =         "yes";
+    fraction deviation allowed = 0.920000017;

```

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+Phase Corr Algorithm = "No";
+Single Spur Suppression = "no";
+Change RC Noise Clip = "no";
Slice Offc. AP (P=+mm) = 1.45663166;
                RL (L=+mm) = -2.91326213;
                FH (H=+mm) = 29.1325607;
    Ang. AP (deg) = 0;
        RL (deg) = -0;
        FH (deg) = -0;
    Free rotatable = "no";
Shim Size AP (mm) = 45;
        RL (mm) = 45;
        FH (mm) = 106.666664;
    Offc. AP (P=+mm) = -35.500309;
        RL (L=+mm) = 32.9406395;
        FH (H=+mm) = 7.61735725;
    Ang. AP (deg) = 0;
        RL (deg) = -3.74499471e-17;
        FH (deg) = -0;
Extended Function. Opt. = "28";
Total scan duration = "13:11.5";
Rel. SNR = 24.8585014;
Act. TR (ms) = "11640";
Act. TE (ms) = "7.9";
ACQ matrix M x P = "100 x 100";
ACQ voxel MPS (mm) = "2.00 / 2.00 / 6.00";
REC voxel MPS (mm) = "1.56 / 1.56 / 6.00";
Scan percentage (%) = 100;
WFS (pix) / BW (Hz) = "1.394 / 726.7";
TSE factor = 50;
TSE es / shot (ms) = "4.0 / 206";
TEeff / TEequiv (ms) = "8 / 7 ";
Head SAR = "< 100 %";
Head SAR / level = "< 3.2 W/kg / normal";
SED = "< 1.4 kJ/kg";
Coil Power = "29 %";
Max B1rms = "0.97 uT";
PNS / level = "46 % / normal";
dB/dt = "94.9 T/s";
Sound Pressure Level (dB) = 31.935873;
VBR initial level = 0.430231363;
VBR gradient reduction = 1;

```