Magnetic Resonance Spectroscopy Investigations of Alzheimer Disease

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Graduate Program in Medical Biophysics  
A thesis submitted in partial fulfillment of the requirements for the degree in Doctor of Philosophy  
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Magnetic Resonance Spectroscopy Investigations of Alzheimer Disease

(Thesis format: Integrated article)

by

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Graduate Program in Medical Biophysics
Schulich School of Medicine and Dentistry

A thesis submitted in partial fulfillment
of the requirements for the degree of
Doctor of Philosophy

The School of Graduate and Postdoctoral Studies
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Abstract

Alzheimer disease is a progressively devastating neurodegenerative disease of the brain that impairs cognition and is ultimately fatal. Cholinesterase inhibitors are the current standard treatment for Alzheimer disease and they can alleviate some of the symptoms and thus improve quality of life. Cognitive measures aid in the diagnosis and monitoring of individuals with Alzheimer disease, but they do not directly measure disease pathophysiology. The purpose of this thesis is to investigate metabolic changes measured with proton magnetic resonance spectroscopy within the hippocampus and posterior cingulate, two brain regions known to be effected in Alzheimer disease, following cholinesterase inhibitor treatment. Such treatment is aimed at increasing the deficit of acetylcholine in Alzheimer disease. Secondly, to develop a 7 Tesla proton magnetic resonance spectroscopy data acquisition and metabolite quantification protocol to be used for future studies.

In one study, proton magnetic resonance spectroscopy at 4 Tesla was used to measure the effects of four months of galantamine treatment (a cholinesterase inhibitor). An increase in the excitatory neurotransmitter glutamate was detected in the right hippocampus, and was associated with increased cognitive performance. In a second study, proton magnetic resonance spectroscopy at 3 Tesla was used to measure the effects of rivastigmine (a second cholinesterase inhibitor). The ratio of the neuronal marker N-acetylaspartate to creatine was decreased in the bilateral posterior cingulate cortex, which was associated with cognition.
Finally, a quantitative proton magnetic resonance spectroscopy protocol at 7 Tesla was developed that incorporates subject-specific macromolecule removal. Absolute *in vivo* metabolite concentrations measured were in agreement with previous studies, and this protocol is ideal for applications in diseased conditions where macromolecule contributions may deviate from the norm.

**Keywords:** Alzheimer disease, cholinesterase inhibitors, galantamine, glutamate, high magnetic field, macromolecule removal, Magnetic Resonance Spectroscopy, metabolite quantification, *N*-acetylaspartate, rivastigmine
Co-Authorship

The following thesis contains material from previously published manuscripts and conference presentations, as well as material from a third manuscript that has been accepted for publication. Permission was obtained from the publishers to reproduce each manuscript, and appear in Appendix A. The contributions of co-authors for each chapter are summarized below. With the exception of the co-author contributions listed below, Jacob Penner performed all of the development, data acquisition and analysis, and preparation of manuscripts.

The material contained in Chapter 2 has been published in Progress in Neuropsychopharmacology & Biological Psychiatry in a manuscript entitled ‘Increased glutamate in the hippocampus after galantamine treatment for Alzheimer disease’ (2010; 34: 104-110). Chapter 2 also contains material presented at two international and two national conferences in 2008 as follows: at the Alzheimer’s Association International Conference on Alzheimer’s Disease (2008), at the Annual Meeting of the International Society of Magnetic Resonance in Medicine (2008), at the Canadian Association on Gerontology’s 37th Annual Scientific & Educational Meeting (2008), and at the Annual Meeting of the Canadian Geriatrics Society (2008). Co-authors were Jacob Penner, Raul Rupsingh, Matthew Smith, Jennie L. Wells, Michael J. Borrie, and Robert Bartha. Raul Rupsingh was involved in data acquisition and development of quantification steps. M. Smith was involved in the design and management of the study. J.L. Wells, and M.J. Borrie recruited patients and performed clinical tests. M.J.
Borrie and R. Bartha were involved in the design of the study. R. Bartha provided guidance and support in the preparation of both the manuscript and conference presentations.

The material contained in Chapter 3 has been accepted to Dementia and Geriatric Cognitive Disorders in a manuscript entitled ‘Reduced $N$-acetylaspartate to creatine ratio in the posterior cingulate correlates with cognition in Alzheimer disease following four months of rivastigmine treatment’ (accepted August 19, 2014). Chapter 3 also contains material presented at the 6th Canadian Conference on Dementia (2011) and at the Alzheimer’s Association International Conference (2012). Co-authors were Jacob Penner, Jennie L. Wells, Michael J. Borrie, Matthew Smith, Sarah M. Woolmore-Goodwin, and Robert Bartha.

J.L. Wells, and M.J. Borrie recruited patients and performed clinical tests. M. Smith was involved in the design and management of the study. S.M. Woolmore-Goodwin was involved in managing subjects and clinical data. M.J. Borrie and R. Bartha were involved in the design of the study. R. Bartha provided guidance and support in the preparation of the manuscript.

The material contained in Chapter 4 has been published in Magnetic Resonance in Medicine in a manuscript entitled ‘Semi-LASER $^1$H MR Spectroscopy at 7 Tesla in Human Brain: Metabolite Quantification Incorporating Subject-Specific Macromolecule Removal’ (2014; Epud ahead of print. doi: 10.1002/mrm.25380). Chapter 4 also contains material presented at the Annual Meeting of the International Society of Magnetic Resonance in Medicine (2010, 2011 & 2014). Co-authors were Jacob Penner and Robert Bartha. In addition, co-authors for portions of data presented at the meetings listed were Andrew Curtis, Andrew Lim, Kyle Gilbert, Martyn Klassen, and Joseph Gati. The $B_{1+}$ shimming protocol was developed by A. Curtis. A. Lim assisted with the development of the metabolite prior-
knowledge fitting template. K. Gilbert developed the radiofrequency coils used to acquire all MRI imaging and spectroscopic data. M. Klassen and J. Gati provided hardware and data management assistance. R. Bartha was involved provided guidance and support in the preparation of the manuscript.
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deciding to move our family to Canada, in so doing you gave me the educational opportunities I have had. Your lifetime of hard physical work provided for your family. There is no doubt that I have inherited my particular attention to detail from you.

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<th>Description</th>
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<tr>
<td>$^1$H</td>
<td>Proton</td>
</tr>
<tr>
<td>$^{11}$C</td>
<td>Carbon-11</td>
</tr>
<tr>
<td>$^{18}$F</td>
<td>Fluorine-18</td>
</tr>
<tr>
<td>Δ</td>
<td>Change in</td>
</tr>
<tr>
<td>$\omega_0$</td>
<td>Larmor frequency</td>
</tr>
<tr>
<td>Aβ</td>
<td>$\beta$-amyloid</td>
</tr>
<tr>
<td>ACh</td>
<td>Acetylcholine</td>
</tr>
<tr>
<td>AChE</td>
<td>Acetylcholinesterase</td>
</tr>
<tr>
<td>AD</td>
<td>Alzheimer disease</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>Alzheimer disease assessment scale-cognitive subscale</td>
</tr>
<tr>
<td>AHP</td>
<td>Adiabatic half passage pulse</td>
</tr>
<tr>
<td>AFP</td>
<td>Adiabatic full passage pulse</td>
</tr>
<tr>
<td>$B_0$</td>
<td>Static magnetic field of MR system</td>
</tr>
<tr>
<td>$B_1$</td>
<td>Magnetic field from radiofrequency coil</td>
</tr>
<tr>
<td>$Ca^{2+}$</td>
<td>Calcium-2+ atom</td>
</tr>
<tr>
<td>ChEi</td>
<td>Cholinesterase inhibitors</td>
</tr>
<tr>
<td>Cho</td>
<td>Choline</td>
</tr>
<tr>
<td>CNS</td>
<td>Central nervous system</td>
</tr>
<tr>
<td>Cr</td>
<td>Creatine</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebral spinal fluid</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>ECC</td>
<td>Eddy current correction</td>
</tr>
<tr>
<td>FDG</td>
<td>Fluorodeoxyglucose</td>
</tr>
<tr>
<td>FID</td>
<td>Free induction decay</td>
</tr>
<tr>
<td>FT</td>
<td>Fourier transform</td>
</tr>
<tr>
<td>Glu</td>
<td>Glutamate</td>
</tr>
<tr>
<td>Gln</td>
<td>Glutamine</td>
</tr>
<tr>
<td>Glx</td>
<td>Glutamate + glutamine</td>
</tr>
<tr>
<td>GM</td>
<td>Grey matter</td>
</tr>
<tr>
<td>GPC</td>
<td>Glycerolphosphocholine</td>
</tr>
<tr>
<td>HSVD</td>
<td>Hankel singular value decomposition</td>
</tr>
<tr>
<td>LASER</td>
<td>Localization by adiabatic selective refocusing</td>
</tr>
<tr>
<td>MCI</td>
<td>Mild cognitive impairment</td>
</tr>
<tr>
<td>mI</td>
<td>Myo-inositol</td>
</tr>
<tr>
<td>mM</td>
<td>milli-moles/litre</td>
</tr>
<tr>
<td>MMSE</td>
<td>Mini-mental state examination</td>
</tr>
<tr>
<td>MoCA</td>
<td>Montreal cognitive assessment</td>
</tr>
<tr>
<td>MR</td>
<td>Magnetic resonance</td>
</tr>
<tr>
<td>MRI</td>
<td>Magnetic resonance imaging</td>
</tr>
<tr>
<td>MRS</td>
<td>Magnetic resonance spectroscopy</td>
</tr>
<tr>
<td>MTL</td>
<td>Medial temporal lobe</td>
</tr>
<tr>
<td>NAA</td>
<td>N-acetylaspartate</td>
</tr>
<tr>
<td>nAChR</td>
<td>Nicotinic acetylcholine receptors</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Full Form</td>
</tr>
<tr>
<td>--------------</td>
<td>-----------</td>
</tr>
<tr>
<td>NFT</td>
<td>Neurofibrillary tangle</td>
</tr>
<tr>
<td>NMDA</td>
<td>N-methyl-D-aspartate</td>
</tr>
<tr>
<td>PET</td>
<td>Positron emission tomography</td>
</tr>
<tr>
<td>PC</td>
<td>Phosphocholine</td>
</tr>
<tr>
<td>PCr</td>
<td>Phosphocreatine</td>
</tr>
<tr>
<td>PiB</td>
<td>Pittsburgh compound B</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts-per-million</td>
</tr>
<tr>
<td>PRESS</td>
<td>Point resolved spectroscopy</td>
</tr>
<tr>
<td>RF</td>
<td>Radiofrequency</td>
</tr>
<tr>
<td>QUALITY</td>
<td>Quantification improvement by converting lineshapes to the lorentzian type</td>
</tr>
<tr>
<td>QUECC</td>
<td>Combined QUALITY deconvolution and ECC</td>
</tr>
<tr>
<td>SAR</td>
<td>Specific absorption rate</td>
</tr>
<tr>
<td>semi-LASER</td>
<td>Semi-localization by selective refocusing</td>
</tr>
<tr>
<td>STEAM</td>
<td>Stimulated echo acquisition mode</td>
</tr>
<tr>
<td>T₁</td>
<td>Longitudinal relaxation time constant</td>
</tr>
<tr>
<td>T₂</td>
<td>Transverse relaxation time constant</td>
</tr>
<tr>
<td>tCho</td>
<td>Total choline</td>
</tr>
<tr>
<td>tCr</td>
<td>Total creatine</td>
</tr>
<tr>
<td>TE</td>
<td>Echo time</td>
</tr>
<tr>
<td>TI</td>
<td>Inversion time</td>
</tr>
<tr>
<td>TI₁</td>
<td>Double-inversion recovery time one</td>
</tr>
<tr>
<td>TI₂</td>
<td>Double-inversion recovery time two</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
<td>-------------</td>
</tr>
<tr>
<td>TR</td>
<td>Repetition time</td>
</tr>
<tr>
<td>TSP</td>
<td>Sodium 3-trimethylsilyl-propionic acid</td>
</tr>
<tr>
<td>VAPOR</td>
<td>Variable pulse power and optimized relaxation delays</td>
</tr>
<tr>
<td>WM</td>
<td>White matter</td>
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</table>
Chapter 1

Introduction

The goal of this thesis is to use proton magnetic resonance spectroscopy (\(^1\text{H} \text{ MRS}\)) to characterize metabolite changes in the brain following cholinesterase inhibitor (ChEI) treatment in people with Alzheimer disease (AD). Further, a short echo-time \(^1\text{H} \text{ MRS}\) acquisition and metabolite quantification scheme is to be developed for use in humans at a magnetic field strength of 7 Tesla (T). This chapter introduces the neuropathology, diagnosis, and treatment of AD along with current imaging biomarkers under development to monitor disease status and progression. This chapter also describes the magnetic resonance (MR) theory and MRS methodology relevant to the proceeding chapters.
1.1 Alzheimer disease

1.1.1 Overview

Alzheimer disease (AD) is a progressive and neurodegenerative disease of the brain and is the most common cause of dementia. AD is symptomatically characterized by progressive loss in memory, language, executive, and visuospatial function, an inability to perform acts of daily living, and altered personality or mood. It therefore has a profound impact on those diagnosed with the disease and their caregivers. There is currently no known cure for AD, with death occurring approximately seven to ten years after it is clinically evident (Alzheimer’s Society of Canada, January 2009). The Alzheimer’s Society of Canada estimates that half a million Canadians have AD, and 24 million worldwide (January 2009). With the aging global population and the fact that age is a risk factor for AD, this disease will become an even greater burden to our health care system in the coming decades.

1.1.2 Neuropathology

The neuropathology of AD was first described in 1906 by German psychiatrist and neuropathologist Alois Alzheimer (1), after whom the disease was named. Alois Alzheimer first identified numerous senile plaques and densely twisted bundles of neurofibrils on post-mortem histological tissue stains from an individual who suffered from a unique progressive dementia (2). These have more recently been identified as β-amyloid (Aβ) plaques and neurofibrillary tangles (NFTs) and are the key pathological hallmarks of AD. Aβ plaques and NFTs are abnormal accumulations of two separate misfolded proteins. Aβ plaques are found
outside of neurons (extracellular) (3) and consist of aggregated Aβ peptides of 40-42 amino acids (4). Aβ plaques are in close proximity to nerve cells and are neurotoxic. Neurofibrillary tangles form inside neurons (intracellular) and are an accumulation of hyperphosphorylated tau protein (5). Tau is a protein that aids in the maintenance of microtubules, which provide support to the neuron.

Other neuropathological effects of AD include increased microglial and astroglial cell activation and significant brain tissue atrophy. Further, in AD there is a reduction in acetylcholine (ACh), which is a neurotransmitter of the central nervous system (CNS). The pathological alterations observed in AD start in the paralimbic and medial temporal lobe (MTL) structures, including the hippocampus and entorhinal cortex, and later move into the neocortical association areas (6). Eventually, the gradual cortical atrophy caused by neuronal dysfunction and decreased synaptic connectivity results in cognitive and executive function deficits (7,8).

### 1.1.3 Diagnosis and clinical assessments

The National Institute of Neurological and Communicative Disorders and Stroke and the Alzheimer’s Disease and Related Disorders Association (NINCDS-ADRDA) formulated criteria for the definition of Alzheimer disease in 1984, and these are commonly used for the diagnosis of AD (9). These criteria lead to a diagnosis of probable AD based on cognitive impairment with suspected dementia backed up by neuropsychological evaluation. The impaired cognitive domains include: memory, language (aphasia), perceptual skills (agnosia), attention, constructive abilities, orientation, problem solving, and functional abilities (9).
Family history may also help in the diagnosis of AD.

Cognitive assessments, designed to evaluate cognitive performance, are predominantly used to diagnose and monitor the clinical progression of AD. The most common clinical cognitive test is the Mini-Mental State Examination (MMSE), which consists of eleven items designed to evaluate 5 domains: memory, orientation, attention, language, and motor skills (10). MMSE scores range from a value of 30, indicating no impairment, to a value of 0, indicating profound impairment. A second widely used clinical trials cognitive test and primary cognitive outcome measure, is the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog), which consists of seven performance items and four clinical rating items covering memory, orientation, and language (11). ADAS-cog score values range from 0, no impairment, to a value of 70, severe impairment. The MMSE and ADAS-cog offer modest sensitivity to AD (11-13), but lack specificity (11-13). The Montreal Cognitive Assessment (MoCA) is another clinical cognitive test developed for use in people with mild cognitive impairment (MCI) and early AD (14). The MoCA is therefore more sensitive in early AD, but is not yet as commonly used in clinical evaluation despite numerous studies showing its high sensitivity in the detection of MCI and AD (15-23). The MoCA assesses 8 domains: attention and concentration, executive functions, memory, language, visuoconstructional skills, conceptual thinking, calculations, and orientation, with a perfect score equal to 30.

Complicating the differential diagnosis of AD are other forms of dementia, including vascular dementia, dementia with Lewy bodies, and frontotemporal dementia, often presenting with overlapping symptoms. Furthermore, other causes of cognitive decline
include hematoma, normal pressure hydrocephalus, tumour, and stroke (24). As a result, Alzheimer disease clinical diagnostic criteria result in specificity between 76% and 88% and sensitivity between 53% and 65% (25), and therefore there is room for improvement. Currently a definitive diagnosis of AD can only be made upon post-mortem histopathological confirmation (6). The diagnostic accuracy of AD may be improved with the inclusion of cerebral spinal fluid (CSF) biomarkers and neuroimaging markers.

1.1.4 Treatment

Significant research resources are being allocated to the development of disease modifying therapies that could slow or prevent further progression of Alzheimer disease (24), however no such therapies are currently available. The standard of care for patients with AD is treatment with a cholinesterase inhibitor (ChEI) (26,27).

Reduced levels of the neurotransmitter acetylcholine (ACh) in AD contributed to the formation of the cholinergic hypothesis (28), which proposes that AD involves reduced synthesis of acetylcholine and in turn leads to reduced cholinergic neurotransmission (29,30). Pharmaceuticals known as cholinesterase inhibitors (ChEI) have been developed to improve cholinergic neurotransmission by inhibiting the breakdown of ACh within the synaptic cleft by the enzyme acetylcholinesterase (AChE). Currently in clinical use are rivastigmine, donepezil, and galantamine, which are all second-generation ChEIs and have been proven to improve cognition with similar cognitive efficacy in a meta-analysis of all three drugs (31). However, cognitive improvements with ChEI treatment are only temporary because they do not modify the underlying disease process.
Memantine is the only clinically approved drug for the treatment of AD that does not act on the cholinergic pathway, but rather it is a $N$-methyl-D-aspartate (NMDA) receptor antagonist. Over stimulation of NMDA receptors can lead to an excess of Ca$^{2+}$ ions within the neuron, potentially leading to cell death; also known as excitotoxicity. Memantine inhibits excitation of NMDA receptors and therefore reduces excitotoxity.

### 1.2 Neuroimaging of Alzheimer disease

The diagnosis of Alzheimer disease is currently based on clinical evaluations and patient histories and neuroimaging is used to rule out other diseases with similar cognitive deficits, such as hematoma, normal hydrocephalus, brain glioma, and stroke (24). Further, neuroimaging is a promising area of research because it can provide quantitative measures of the underlying disease pathology, beyond clinical cognitive endpoints. Alzheimer’s disease is being studied with many different neuroimaging techniques in an effort to find biomarkers of disease progression that are sensitive and specific early in the disease process. Such biomarkers will improve diagnosis and monitoring of disease status. Early diagnosis will become vital when disease-modifying therapies are available, allowing for intervention before the pathological processes of amyloid protein plaque formation and tau protein neurofibrillary tangle creation is too advanced (6,32).
1.2.1 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) uses only non-ionizing radiation and is capable of producing high-resolution structural images (~1 mm\(^3\) voxel size) with excellent soft tissue contrast. This capability is due in part to the high water content of soft tissue (~56 mol/L), as water molecules contain protons that are used to generate the \textit{in vivo} MRI signal. Longitudinal MRI studies have shown structural changes in Alzheimer disease including decreased volume of the hippocampus (33-36), in one study distinguishing normal controls from AD with 93.3% specificity and 80.6% sensitivity (36). Studies have also shown increased volume of the fluid-filled spaces known as the ventricles (37), and global atrophy (38-40), where one study reported 91% specificity and 84% sensitivity in classifying control versus AD (40). However, these structural changes may also occur in other forms of dementia and therefore their specificity is unclear. As a result, they are often used in combination with cognitive measures.

1.2.2 Positron Emission Tomography

Positron Emission Tomography (PET) is an imaging modality that detects and creates images from nuclides that emit positrons, which in turn release two gamma rays in opposite direction. The two gamma rays interact with the PET detectors at two different locations and this information is used to determine where they originated. Positron-emitting nuclides can be attached to naturally occurring molecules and are known as radiotracers, which can then be injected intravenously to create images of targeted biological processes.

In Alzheimer disease there are two radiotracers that have been most widely studied.
First is fluorodeoxyglucose (FDG), which consists of a positron emitting $^{18}\text{F}$-labeled deoxyglucose. FDG travels throughout the body and is found in higher concentrations in regions with heightened glucose energy metabolism. FDG-PET is therefore used to image glucose metabolism and has shown widespread decreased metabolism in the brains of AD patients, especially in the posterior cingulate, temporoparietal cortex, and prefrontal cortex (41-43). A previous study reported 51.5% specificity at 80% sensitivity in discriminating health controls from those that converted to MCI or AD based on medial temporal and parietal FDG-PET (44). Second, is Pittsburgh compound B ([$\text{[N-Methyl-}^{11}\text{C}]_2(4'$-methylaminophenyl)-6-hydroxybenzothiazole), which is labeled with a positron emitting $^{11}\text{C}$. PiB binds to β-amyloid plaques and studies have shown heightened cortical PiB retention in the brains of AD patients (45-48), and a previous study reported 94.4% specificity and 94.4% sensitivity in discriminating controls from AD based on PiB binding in the precuneus (48). More recently, multiple $^{18}\text{F}$ amyloid binding agents (florbetapir, florbetaben, NAV4694) have shown promise in detecting fibrillary amyloid. The major advantage of $^{18}\text{F}$ compounds over PiB is due to the longer radioactive half-life of $^{18}\text{F}$ (110 mins) compared to $^{11}\text{C}$ (20 mins), negating the need for an on-site cyclotron and radiochemistry expertise (49). A limitation of PET is that radiotracers contain radioactive isotopes and therefore they deposit ionizing radiation into the body. For example, a typical brain FDG scan results in an effective radiation dose of 14 mSv (50), compare that to 2 mSv for a typical head CT scan (50) and the average annual dose from background radiation of 3 mSv/year in the United States (50). However, a PET scan may aid in diagnosis and therefore the benefits may outweigh the radiation risk.
1.2.3 Magnetic Resonance Spectroscopy

Metabolic alterations induced by Alzheimer disease pathological processes are thought to precede detectable structural and cognitive changes, and therefore metabolite levels may provide early biomarkers. Proton (\(^1\)H) MRS is a non-invasive imaging technique capable of directly detecting mobile metabolites in the human brain with concentrations greater than approximately 0.5 mmol/L (mM) (51). Typical in vivo brain metabolite concentrations range from 1 to 15 mM. The most abundant metabolites, and therefore most easily measured by \(^1\)H MRS, in the human brain are N-acetylaspartate, glutamate, creatine-containing compounds, choline-containing compounds, and myo-inositol.

1.2.3.1 N-acetylaspartate

N-acetylaspartate (NAA) is an amino acid found primarily within neurons in the human brain, and the concentration of NAA within neurons is roughly one thousand times the concentration in the extracellular space (52). More specifically, 75% of the NAA content within a neuron is found in the cytosol and 25% in the mitochondria (52). The fact that essentially all of the NAA is within neurons has lead to an interest in relating NAA levels to changes in neurological diseases. NAA levels are often equated to neuronal density or viability. Previous studies have reported decreased NAA in subjects with AD compared to elderly controls in the parietal and occipital cortex (53), gray matter (54,55), hippocampus (56-58), and posterior cingulate (59). Further, reversible changes in NAA levels have been reported following treatment in neurological disorders, suggesting that NAA is reduced before cell death occurs. This decline could be explained by reversible mitochondrial
dysfunction, as NAA synthesis has been linked to mitochondrial activity \textit{in vitro} (60) and the pathogenesis of neurodegeneration often involves mitochondrial dysfunction (61). Therefore, NAA depletion may represent a combination of reduced neuronal density and impaired neuronal function. Therefore, NAA may be an indicator of therapeutic efficacy in AD.

The multifaceted role of NAA is not entirely understood but it has been shown that glucose metabolism is linked to the production of NAA (62), and therefore NAA synthesis is related to energy metabolism within neurons. This relationship is further supported by the fact that glucose is a source for acetate and aspartate, the constituents of NAA. Further, the hydrolysis of NAA into acetate and aspartate, and subsequent active transport out of the neuron, acts as a molecular water pump to maintain a higher extracellular concentration of water (63).

The concentration of NAA in the human brain is approximately 10 mM, which makes it one of the most abundant metabolites in human brain. NAA is easily identified in \textit{in vivo} \textsuperscript{1}H MRS spectra because of its larger single peak at 2.01 ppm.

\subsection{1.2.3.2 Glutamate}

Glutamate (Glu) is the most abundant excitatory neurotransmitter of the central nervous system. Glu is stored in vesicles and is released from the pre-synaptic neuron during neurotransmission and can subsequently bind to post-synaptic N-methyl-D-aspartate (NMDA) receptors. When Glu binds to a NMDA receptor, ions such as \(\text{Ca}^{2+}\) flow into the postsynaptic neuron. Glu excitotoxicity is a feature of AD (64) and occurs when excess Glu accumulates in the synaptic cleft binding to NMDA receptors and causing an excess influx of
Ca$^{2+}$, and the excessively high intracellular Ca$^{2+}$ concentration leads to mitochondrial damage (64). In healthy brain tissue glutamate transporters primarily found on membranes of astrocytes (a type of glial cell) take up Glu, and within the astrocyte glutamine synthetase converts Glu into its precursor glutamine (Gln) (65). Gln is then transported across the extracellular space back into the presynaptic neuron where it is synthesized into Glu, thereby avoiding excess Glu accumulation in the extracellular space (65). Previous studies have reported decreased Glx (Glu + Gln) in subjects with AD compared to elderly controls in occipital gray matter (54), in the cingulate cortex (66), and in the posterior cingulate gyrus and precuneus (67). Thus, it is becoming increasingly important to understand how Glu could be used as a biomarker for AD.

The concentrations of Glu and Gln in the human brain are approximately 8-10 mM and 2-3 mM, respectively. Glu and Gln produce J-coupled multiplets in the in vivo $^1$H MRS spectrum in the range of 2.0-2.5 ppm. Separating the overlapping peaks from Glu and Gln is challenging and sometimes infeasible, especially at magnetic fields strengths equal to or below 3 Tesla, and therefore the sum of Glu and Gln (Glx) is often reported in the literature.

1.2.3.3 Creatine

The sum of creatine (Cr) and phosphocreatine (PCr) is referred to as total Cr (tCr) in this thesis, and Cr and PCr are in constant exchange (68). More specifically, PCr serves as a reserve for high energy phosphates in the cytosol and buffers cellular ATP/ADP reservoirs (69). PCr is therefore used as an energy marker of both neurons and astrocytes (68).

The total concentration of Cr plus PCr in the human brain is approximately 8-9 mM
and both Cr and PCr produce strong single peaks at ~3.0 and ~3.9 ppm, which are easily identified in in vivo $^1$H MRS spectra. Measurement of metabolite levels from MRS often uses tCr as a normalization reference. This approach is used because tCr levels have been shown to be fairly stable within an individual over time (54), and because taking the ratio of two metabolites eliminates the need for tissue partial volume corrections and results in a unit-less quantity that is easy to compare between scanners and research laboratories.

1.2.3.4 Choline

Glycerolphosphocholine (GPC) and phosphocholine (PC), which are commonly summed and reported as Cho (GPC + PC) in the literature and in this thesis, are essential for membrane lipid synthesis (68). Increased Cho is thought to reflect higher membrane turnover resulting in the increased release of water soluble Cho containing compounds from the cell membrane (68). Increased Cho has been reported in AD in bilateral posterior cingulate gyri and inferior precunei (70), as compared to elderly controls. It has been theorized that an increase in Cho can also result from a compensatory reaction to low levels of acetylcholine due to cholinergic neuronal death in AD (71).

The total concentration of Cho (PC + GPC) in the human brain is approximately 1-2 mM, which is lower than NAA, Glu, and tCr. However, Cho produces a strong single peak at ~3.2 ppm and therefore is nonetheless easily identified in in vivo $^1$H MRS spectra.
1.2.3.5 myo-Inositol

Myo-inositol (mI) is a cyclic sugar alcohol produced by the human body from glucose, and plays an important role as the structural basis for a number of secondary messengers in eukaryotic cells (72). Myo-inositol is thought to be a marker of gliosis, which is the proliferation of glial cells, partly because glial cells are known to have a high concentration of mI (73). Gliosis is often associated with neuronal atrophy or inflammation of neural tissue. Increased mI has been reported in AD in parietal and occipital cortex (53), bilateral posterior cingulate gyri and inferior precunei (70), gray matter (54), and bilateral posterior cingulate (59). This increase in mI may be indicative of the aggregation of glial cells. Another theory is that the conversion of mI into phosphatidylinositol, used in neural membranes, is inhibited in AD, causing a build-up of mI and a depletion of phosphatidylinositol (74).

The concentration of mI in the human brain is approximately 5-6 mM, and mI produces a strong, easily identified multiplet centred at ~3.5 ppm in \textit{in vivo} $^1$H MRS spectra.

1.2.3.6 MRS metabolite measurement for treatment efficacy

$^1$H MRS metabolite level measurement is an excellent potential biomarker of treatment efficacy due to a number of reasons. Firstly, $^1$H MRS is a non-invasive imaging method that yields \textit{in vivo} metabolite concentrations and can easily be added to standard imaging protocols without affecting other imaging measures or cognitive testing. Secondly, metabolite levels are quantitative measures of chemical species, and therefore are not affected by subject performance, mood, or wakefulness. Further, metabolic alterations caused by Alzheimer disease are thought to precede detectable structural and cognitive changes,
potentially allowing for earlier detection and earlier response to treatment. More specifically
with beneficial treatment: NAA can be used as a biomarker of neuronal density or function
and should increase to normal levels, Glu can be used to measure excitatory
neurotransmission that should increase to normal levels, Cho can be used to measure cellular
turnover or regrowth and should decrease to normal levels, and mI can be used to measure
atrophy or inflammation of neurons (associated with gliosis) and should decrease to normal
levels.

1.3 Principles of Magnetic Resonance

The studies detailed in this thesis utilize proton magnetic resonance (MR) imaging and
MR spectroscopy to measure metabolites in the human brain, and therefore the basic theory
of magnetic resonance will be provided in this section. Symbols with an arrow on top
represent vector quantities that have a magnitude and a direction.

1.3.1 Magnetization

Nuclear magnetic resonance can be described classically or quantum mechanically. The
concept of quantum spin can only be described by quantum mechanics, but classical
principles will be used to explain the behaviour of the macroscopic magnetization.

The phenomenon of nuclear magnetic resonance occurs when a nucleus with a
magnetic moment ($\mu$) is placed in a magnetic field ($B$). The magnetic field will exert a
rotational force, or torque ($\tau$), on the magnetic moment given by the following cross-
product:
\[ \mathbf{\tau} = \mathbf{\mu} \times \mathbf{B} = \mu B \sin \theta \quad (1.1) \]

where \( \theta \) is the angle between the two vectors. The direction of the torque is normal to the plane defined by \( \mu \) and \( B \), leading to precession of the nucleus about \( B \). Further, the torque is zero when \( \mu \) is either parallel or anti-parallel to \( B \) as \( \sin(0^\circ) = \sin(180^\circ) = 0 \), and therefore precession only occurs when \( \mu \) and \( B \) are not in the same direction. The angular frequency of the precession is known as the Larmor frequency \( (\omega_0) \) and is given by:

\[ \omega_0 = \gamma B_0 \quad (1.2) \]

A magnetic moment in a magnetic field has potential energy \( E \) defined by the following dot-product:

\[ E = -\mathbf{\mu} \cdot \mathbf{B} = -\mu B \cos \theta \quad (1.3) \]

The convention in MRI is to set the z-direction along \( B \), such that \( \mathbf{B} = B_0 \hat{z} \). Since \( B_0 \) is only in the z-direction, the dot-product in Equation 1.2 yields:

\[ E = -\mu_z B_0 \quad (1.4) \]
where \( \mu_z \) and \( B_0 \) are in the z-direction.

The magnitude of the spin angular momentum (\( S \)) of a nucleus is given by:

\[
S = \hbar \sqrt{s(s + 1)} \tag{1.5}
\]

where \( s \) the associated quantum number with values equal to 0, 1/2, 1, 3/2, 2, ..., and \( \hbar \) is Planck’s constant divided by \( 2\pi \). Therefore, nuclei with a non-zero spin quantum number have a non-zero spin angular momentum and an associated magnetic moment:

\[
\mu = \gamma S \tag{1.6}
\]

where \( \gamma \) is the gyromagnetic ratio that is unique to each nucleus. Substituting Equation 1.4 into Equation 1.5 gives:

\[
\mu = \gamma \hbar \sqrt{s(s + 1)} \tag{1.7}
\]

therefore the magnetic momentum is quantized. The discrete set of possible \( z \) components of the magnetic moment is given by another quantum number, \( m \):

\[
\mu_z = \gamma \hbar m \tag{1.8}
\]
and there are $2s + 1$ values of $m$ given by $-s, s + 1, \ldots, s - 1, s$. Substituting Equation 1.7 into Equation 1.3 yields:

$$E = -\gamma \hbar m B_0$$

(1.9)

the $2s + 1$ values for the potential energy of the magnetic moment in a magnetic field.

The probability ($P_m$) that a nucleus is in the quantum energy state $m$ is given by the Boltzmann distribution:

$$P_m = \frac{N_m}{N} = \frac{e^{-E/kT}}{Z}$$

(1.10)

where $N_m$ is the number of nuclei in state $m$, $N$ is the total number of nuclei, $k$ is the Boltzmann constant ($1.3805 \times 10^{-23}$ Joules/Kelvin), $T$ is the temperature in Kelvin, and $Z$ is the partition function for an ensemble of spins. The equilibrium longitudinal magnetization ($M_0$) can be derived (75) from the average $\mu_z$ over the total number of nuclei using Equation 1.9 and the fact that $kT \gg \gamma \hbar B_0$ at physiological temperatures:

$$M_0 = \frac{N\gamma^2 \hbar^2 s(s + 1)B_0}{3kT}$$

(1.11)

This net macroscopic magnetization of an ensemble of spins is aligned with the static
magnetic field at thermal equilibrium. There are two reasons for this alignment: (i) magnetic moments aligned with \( B_0 \) are in the lower energy state according to Equation 1.8 and (ii) there is no net transverse magnetization as the spins are randomly distributed (incoherent) in the transverse plane. However, the thermal energy of the system acts to equalize the number of magnetic moments in each energy state, resulting in only a small excess of spins in the lower energy state (on the order of one in a million).

Magnetic resonance scanners are designed to detect magnetization that is perpendicular to the \( B_0 \) direction (in the transverse plane), and therefore the equilibrium magnetization in the \( z \)-direction must be manipulated to produce transverse magnetization that can be detected.

### 1.3.2 Excitation

Excitation refers to the process of generating detectable transverse magnetization \( (M_x = \sqrt{M_x^2 + M_y^2}) \) from the longitudinal magnetization, \( \vec{M} \), and is accomplished by applying a transverse magnetic field (\( \vec{B}_1 \)). The \( \vec{B}_1 \) field applies a torque to \( \vec{M} \), causing it to rotate about \( \vec{B}_1 \) and into the transverse plane by an amount \( \theta \), known as the flip angle:

\[
\theta(t) = \gamma \int_{0}^{\tau} B_1(t) dt \tag{1.12}
\]

where \( \tau \) is the duration of the applied \( B_1 \) field. Since \( \vec{M} \) precesses about \( \vec{B}_0 \) at the Larmor
frequency (Equation 1.2) as it leaves the z-axis, the applied $\vec{B}_1$ must also be precessing at $\omega_0$ to remain perpendicular to $\vec{M}$ as it rotates down into the transverse plane. Figure 1.1 shows the net magnetization rotating into the transverse plane in a frame of reference $(x', y', z')$ that rotates at $\omega_0$ about the $z = z'$ axis. In this rotating frame of reference, $\vec{B}_1$ is stationary and $\vec{M}$ rotates about $\vec{B}_1$ but with no rotation about $z'$.

Figure 1.1: Signal excitation occurs as the net magnetization $\vec{M}$ is rotated by the flip angle $\theta$ about $\vec{B}_1$, producing magnetization in the $x$ and $y$ directions, $M_x$ and $M_y$. 
The gyromagnetic ratio for a proton is 42.576 MHz/T. Human MR systems have static magnetic field strengths \( (B_0) \) on the order of one to several Tesla resulting in precession (Larmor) frequencies of protons in the radiofrequency (RF) range (Equation 1.2). Therefore RF pulses are used to generate the \( B_1 \) fields used for excitation. Conventional RF pulses are applied at a constant frequency (Larmor frequency) with varying amplitude; known as amplitude modulation.

Adiabatic RF pulses, which have benefits at high magnetic field (explained in section 1.4.2), are both frequency and amplitude modulated. When adiabatic RF pulses are played out, they begin with the pulse frequency \( (\omega_{RF}(t)) \) far from the Larmor frequency \( (\omega_0) \). The frequency is then gradually changed over time to match the nuclear precession frequency. The difference between the frequency of the RF pulse and the Larmor frequency is \( \Delta\omega(t) \):

\[
\Delta\omega(t) = \omega_0 - \omega_{RF}(t)
\]

(1.13)

and can be represented by a fictitious field in the z direction. The magnetic field \( (B_1(t)) \) that produces the adiabatic pulse in the transverse plane can be aligned with the \( x' \) axis in a frame of reference that rotates at the instantaneous frequency of the pulse \( (\omega_{RF}(t)) \) about the z axis. In this representation, the \( B_1(t) \) field remains stationary in the reference frame and its amplitude can be modulated as illustrated in Figure 1.2.
Figure 1.2: The effective field \( \vec{B}_{\text{eff}}(t) \) for an adiabatic pulse shown in a frame of reference rotating about the \( z \) axis at \( \omega_{\text{RF}}(t) \). Also shown are the \( \Delta \omega(t)/\gamma \) field arising from the frequency modulation, the amplitude modulated field \( \omega_1(t)/\gamma \), and the angle \( \alpha(t) \) of the rotation of \( \vec{B}_{\text{eff}}(t) \) from the \( z \)-axis.

The total effective field produced, \( \vec{B}_{\text{eff}}(t) \), is given by the vector sum of \( B_1(t) = \omega_1(t)/\gamma \) in the \( x' \) direction and the fictitious field, \( \Delta \omega(t)/\gamma \), in the \( z' \) direction (Figure 1.2). At the beginning of an adiabatic pulse the frequency offset \( \Delta \omega(t) \) is large, \( \Delta \omega(t)/\gamma \gg B_1(t) \), and therefore \( \vec{B}_{\text{eff}}(t) \) is initially along the \( z' \) direction. As time passes \( \omega_{\text{RF}} \) gradually approaches
\( \omega_0 \) and \( \Delta \omega(t) \) approaches zero all while \( B_1(t) \) becomes larger than \( \Delta \omega(t) / \gamma \), until \( B_1(t) \gg \Delta \omega(t) / \gamma \) and \( \tilde{B}_{\text{eff}}(t) \) is swept from being co-linear with \( z' \) to being co-linear with \( x' \) (90° rotation). A 180° sweep can be achieved by gradually changing \( \omega_{\text{RF}} \) from \( \omega_0 \) to a large negative frequency offset, \(-\Delta \omega(t)\), until \(-\Delta \omega(t) / \gamma \gg B_1(t)\) and \( \tilde{B}_{\text{eff}}(t) \) becomes co-linear with \(-x'\). Any magnetization that is collinear with \( \tilde{B}_{\text{eff}}(t) \) at the beginning of the adiabatic pulse is locked and rotated through the frequency sweep. To keep the magnetization locked to \( \tilde{B}_{\text{eff}}(t) \) the adiabatic condition must be fulfilled throughout the frequency sweep, written as:

\[
\gamma \tilde{B}_{\text{eff}}(t) \gg \frac{d\alpha(t)}{dt} \quad (1.14)
\]

where the angle \( \tilde{B}_{\text{eff}}(t) \) makes with the positive \( z \) axis (\( \alpha(t) \)) as illustrated in Figure 1.2 is given by:

\[
\alpha(t) = \arctan \left( \frac{\omega_1(t)}{\Delta \omega(t)} \right) \quad (1.15)
\]
1.3.3 Detection

Following excitation, the magnetization in the transverse plane precesses about the $z$ axis producing a changing magnetic field ($\vec{B}$) in the laboratory reference frame ($x, y, z$). This changing magnetic field creates a changing magnetic flux $\Phi_M$ through a conductive coil, and therefore an electromotive force ($emf$) according to Faraday induction:

$$
emf = -\frac{d\Phi_M}{dt} = -\frac{d}{dt} \int_{\text{area}} \vec{B} \cdot d\vec{a}
$$

where $d\vec{a}$ are the area vectors that are normal to the $z$ axis. The $emf$ creates an alternating current in the coil at the Larmor frequency, which is the detected signal.

1.3.4 Relaxation

The magnetization following an excitation pulse is described by the Bloch Equation (76):

$$
\frac{d\vec{M}}{dt} = \gamma \vec{M} \times \vec{B}_0 - \frac{M_x \vec{x} + M_y \vec{y}}{T_2} - \frac{(M_z - M_0) \vec{z}}{T_1}
$$

where $T_2$ is the transverse relaxation time constant that describes the decay of the transverse magnetization ($M_x$ and $M_y$), and $T_1$ is the longitudinal relaxation time constant that describes the regrowth of the longitudinal magnetization ($M_z$). The Bloch Equation can be separated
into the following components:

\[
\frac{dM_x}{dt} = \gamma M_y B_0 - \frac{M_x}{T_2} \tag{1.18}
\]

\[
\frac{dM_x}{dt} = -\gamma M_y B_0 - \frac{M_x}{T_2} \tag{1.19}
\]

\[
\frac{dM_z}{dt} = -\frac{M_z - M_0}{T_1} \tag{1.20}
\]

and the solutions to Equations 1.18-1.20 are:

\[
M_x(t) = \left(M_x(0)\cos(\omega_0 t) + M_y(0)\sin(\omega_0 t)\right)e^{-\frac{t}{T_2}} \tag{1.21}
\]

\[
M_y(t) = \left(-M_x(0)\sin(\omega_0 t) + M_y(0)\cos(\omega_0 t)\right)e^{-\frac{t}{T_2}} \tag{1.22}
\]

\[
M_z(t) = M_0 + \left(M_z(0) - M_0\right)e^{-\frac{t}{T_1}} \tag{1.23}
\]

where time zero is directly after the excitation pulse has been applied. Over time the excited
magnetization will return to the initial state \( (M_z = M_0 \text{ and } M_{xy} = 0 \text{ as } t \text{ approaches } \infty) \). The regrowth of the longitudinal magnetization \( (T_1 \text{ relaxation}) \) is caused by energy exchange between the excited nuclei and the surrounding lattice, and is therefore called the spin-lattice relaxation time. On the other hand, the loss of phase coherence between nuclei \( (T_2 \text{ relaxation}) \) is caused by energy exchange between spins, and is therefore called spin-spin relaxation. Additional spin dephasing introduced by external field inhomogeneities produces a loss of coherence of the transverse magnetization that is represented by the time constant \( T_2' \). The combined affect of internal spin-spin \( (T_2) \) and external field inhomogeneity \( (T_2') \) relaxation mechanisms is given by the time constant \( T_2^* \):

\[
\frac{1}{T_2^*} = \frac{1}{T_2} + \frac{1}{T_2'}
\]  

(1.24)

Combined Equation 1.21 and Equation 1.22, and replacing \( T_2 \) with \( T_2^* \) yields a simplified equation for the transverse relaxation:

\[
M_{xy}(t) = M_{xy}(0)e^{-\frac{t}{T_2^*}}
\]

(1.25)

This equation represents the MR signal following excitation and is referred to as the free induction decay (FID). Differences in the \( T_1 \) and \( T_2 \) relaxation times of biological tissues are the source of the contrast observed in MR images.
1.3.5 Spatial encoding

Linear gradient magnetic fields are applied in the \( z \) direction (\( B_z \)) to encode the spatial position of spins as follows:

\[
\vec{G}(\vec{r}) = \frac{dB_z}{d\vec{r}}
\]  \hspace{1cm} (1.26)

where \( \vec{r} \) is in the direction of the linear \( B_z \) variation. Applying such a linear gradient field can induce a spatially dependent phase:

\[
\phi(t) = \gamma \int_0^t \vec{G}(\vec{r}, \tau) \cdot \vec{r} d\tau
\]  \hspace{1cm} (1.27)

where \( t \) is the duration of the applied gradient field. This gradient spatial encoding allows the MR signal produced by the object to be associated with its spatial frequency and encoded as a function of k-space, defined as:

\[
\vec{k}_r(t) = \frac{\gamma}{2\pi} \int_0^t \vec{G}(\vec{r}, \tau) d\tau
\]  \hspace{1cm} (1.28)

and therefore:
\[ \phi(t) = 2\pi \vec{k} \cdot \vec{r} \] (1.29)

The k-space signal represents the Fourier transform of the object. Therefore inverse Fourier transform of the k-space data produces the spatial position of the spin signal (the image). Phase encoding gradients can be applied following signal excitation and before data acquisition so that spins accrue a spatially dependent phase as a function of position in the phase-encode direction. Whereas readout gradients are applied during acquisition; the frequency of spins is a function of their position and results in a position dependent phase accrual in the frequency-encode direction.

1.4 Magnetic resonance spectroscopy (MRS)

1.4.1 Water suppression

Most magnetic resonance imaging techniques applied to humans measure the signal from water protons \(^{(1}H\) resonating at a single frequency. The high concentration of water in the human body (e.g. approximately 45 mol/L in human brain grey matter and 40 mol/L in white matter (77)) allows the acquisition of high resolution images in short scan time. Magnetic resonance spectroscopy (MRS) is related technique designed to simultaneously detect multiple signals resonating at different frequencies. Human brain tissue contains numerous mobile metabolites with protons that resonate at specific frequencies depending on their unique chemical environments. In human brain the concentration of water (~50x10\(^3\) mmol/L) is approximately four orders of magnitude larger than the concentrations of
metabolites (~1-10 mmol/L). The large water signal overlaps with the much smaller metabolite signals, making it difficult to identify metabolite peaks. Further, the large difference in signal amplitudes between water and metabolites creates dynamic range problems for the MR system receivers, resulting in baseline distortions that affect metabolite peaks. Therefore, suppression of the signal from water protons is paramount to the reliable detection and quantification of metabolite signals.

The most common approach to suppress the water signal takes advantage of the chemical shift difference between water and metabolite signals, as water resonances at 4.7 ppm and most metabolites of interest resonate between 1.3 and 4.1 ppm (ppm - parts per million frequency shift from a reference standard). Chemical shift selective (CHESS) pulses (78) are most often used to selectively suppress the water signal. This technique uses a selective RF pulse to rotate the longitudinal magnetization of the water protons into the transverse plane (excitation), then a \( B_0 \) magnetic field gradient dephases the magnetization to destroy the water signal. Excitation of the metabolite signals is performed immediately after dephasing the water signal, significantly reducing the contribution of water to the metabolite spectrum.

Ideally, the CHESS pulse will rotate all of the water magnetization by 90° into the transverse plane, so that complete dephasing of the water signal may occur. However, \( B_0 \) and \( B_1 \) inhomogeneities will affect the flip angle leaving some magnetization in the longitudinal direction when the dephasing gradient is applied. The magnetization left in the longitudinal direction will produce a visible signal in the metabolite spectrum. For this reason, in practice multiple CHESS pulses and dephasing gradients are used to destroy the water signal before
metabolite detection. Variable Power and Optimized Relaxation delays (VAPOR) is a common water suppression scheme that uses seven CHESS pulses with specific relative amplitudes and inter-pulse timings to suppress water magnetization equally for flip angles ranging from 65° to 125° (79).

1.4.2 Signal localization

Region specific metabolite levels are of interest in various diseases. MRS localization methods include single voxel spectroscopy and spectroscopic imaging (MRSI); the remainder of this thesis will only deal with single voxel 1H spectroscopy (MRS). A common method used to localize metabolite signals to a specific voxel in the tissue uses three RF pulses applied with orthogonal magnetic field gradients. The two most common 1H MRS sequences are stimulated echo acquisition mode (STEAM) (80) and point resolved spectroscopy (PRESS) (81), which both measure signal echoes. A traditional spin echo is formed when a 180° inversion pulse is applied a duration of time (TE/2, where TE is the echo time) after an excitation pulse. The inversion pulse inverts all spins and causes the spins that have gained and lost phase due to their differences in local magnetic fields to now lose and gain phase, respectively. This results in a refocused signal maximum at TE, called the spin echo.

A STEAM sequence consists of three orthogonal slice selective 90° RF pulses with the following inter-pulse timings (90° - TE/2 - 90° - TM - 90° - TE/2 - echo) where TM is the mixing time. This sequence produces a signal echo called a stimulated echo (82), and its signal originates from the intersection of the three slice selection slabs. The background signals from unwanted echoes are eliminated with crusher gradients applied after each 90°
pulse. There are three advantages of STEAM: (i) very short echo times can be achieved as 90° pulses can be shorter than 180° pulses and TM interval does not contribute to the echo-time, (ii) 90° pulses typically have sharper edge profiles than 180° pulses resulting in more accurate signal localization, and (iii) 90° pulses require less power than 180° pulses, important for SAR considerations, particularly at high magnetic fields. The major disadvantage of STEAM is a 2-fold decrease in the stimulated echo signal as compared to a traditional spin echo. This signal loss occurs because the second 90° pulse only rotates half of the dephased transverse magnetization into the longitudinal direction to be stored there during the TM interval. The other half of the magnetization that remains in the transverse plane is dephased during the TM interval.

A PRESS sequence consists of one 90° RF pulse followed by two 180° RF pulses, applied during orthogonal magnetic field gradients to localize the signal. The first 180° pulse creates a traditional spin echo that is subsequently refocused by the second 180° pulse (as long as the time between the 180° pulses is longer than the time between the 90° pulse and the first 180° pulse), creating the spin echo that is detected. The 180° RF pulses used in PRESS are generally longer than 90° pulses used in STEAM making short TEs more difficult, increasing power deposition, and degrading slice selection profiles. However, the detected spin echo has twice the signal magnitude compared to the STEAM induced stimulated echo, which can be very important due to the inherent low signal intensities of brain metabolites.

Both STEAM and PRESS are susceptible to $B_1$ and $B_0$ inhomogeneities that can lead to non-ideal excitation and incomplete signal refocusing. $B_1$ inhomogeneities cause spins to
experience different $B_1$ fields and therefore different flip angles, whereas $B_0$ inhomogeneities cause dephasing of the spins that can not be refocused by the subsequent refocusing pulses. Also common to STEAM and PRESS protocols is the use of outer volume suppression (OVS) to minimize signals arising from outside the volume of interest. However, OVS can decrease the SNR of metabolites within the measurement volume due to magnetization transfer effects (83).

Localization by adiabatic selective refocusing (LASER) is another MRS localization sequence but unlike STEAM and PRESS, LASER is insensitive to $B_1$ and $B_0$ inhomogeneities. As suggested in the name, LASER utilizes adiabatic RF pulses (84) that are insensitive to $B_1$ and $B_0$ inhomogeneities. Another advantage of adiabatic pulses is their excellent slice selection profiles, sharper than traditional pulses, negating the need for OVS (85). Therefore, the LASER sequence is ideal for in vivo experiments where inhomogeneities are present, and for high field strength applications where achieving $B_1$ uniformity is more challenging. The LASER sequence consists of a global adiabatic half passage (AHP) pulse for non-selective excitation of the entire volume, followed by three pairs of orthogonal slice selective adiabatic full passage (AFP) inversion pulses. Each pair of AFP pulses refocuses the magnetization within a slab, and the three intersecting slabs define the 3D voxel from where the detected signal originates. The phase evolution that occurs during the first AFP in each pair is refocused by the second AFP (85), resulting in a spin echo. Each one of the six AFP pulses in LASER is surrounded by symmetric crusher gradients to destroy any coherent magnetization outside of the selection volume; these crusher gradients help achieve sharp slice profiles. There are two major disadvantages of the LASER sequence. First, longer TEs
are required due to the larger number of pulses. Second, short duration adiabatic pulses require high amplitude RF pulses that may be limited by the maximum available amplifier output and result in higher power deposition potentially exceeding SAR limits. Increasing pulse length is possible to reduce the maximum required B$_1$ at the expense of increasing echo-time.

1.4.3 Chemical shift

Electrons surrounding nuclei are charged particles in motion and therefore create their own magnetic field, resulting in a reduction (or shielding) of the static magnetic field (B$_0$). The altered magnetic field (B) experienced by the nucleus is given by:

$$B = B_0(1 - \sigma)$$

where $\sigma$ is the shielding coefficient, which has dimensionless units of ppm and is independent of B$_0$. The chemical environment determines the electron distribution surrounding the nucleus and subsequently the magnitude of the shielding coefficient. The reduction in magnetic field experienced by the nucleus results in a lower precession frequency, conventionally termed a chemical shift in frequency. The chemical shift ($\delta$) also has dimensionless units of ppm and is given by:

$$\delta = \frac{f - f_{\text{ref}}}{f_{\text{ref}}} \times 10^6$$
where $f$ is the frequency of the nucleus of interest and $f_{\text{ref}}$ is the frequency of a nucleus in a reference compound (i.e. proton MRS commonly uses sodium 3-trimethylsilyl-propionic acid (TSP) as a chemical shift reference). With MRS the unique chemical shifts in frequency produced by electronic shielding are used to identify signals from nuclei in specific molecules.

1.4.4 J-coupling

Some compounds contain nuclear spins that have complex spectral patterns due to a phenomenon known as J-coupling or spin-spin coupling. J-coupling refers to an interaction between non-identical nuclear spins within a molecule through the electrons of covalent bonds, resulting in slight frequency shifts. Figure 1.3a shows the chemical structure of lactate, and Figure 1.3b illustrates the splitting of the peaks from the interaction between methyl and methine protons.
Figure 1.3: The chemical structure (a) and J-coupling spectral pattern (b) for lactate. The one proton on the second carbon (methylene group) and the three protons on the third carbon (methyl group) are J-coupled. The three protons of the methyl group are magnetically equivalent and split the signal from the methine proton into a quartet at 4.2 ppm with a 1:3:3:1 ratio in amplitudes. The single methine proton splits each methyl proton into a doublet at 1.3 ppm with a 1:1 amplitude ratio. Although not shown as such, the total area of the methyl peaks is three times the total area of the methine peaks because the methyl group has three times as many protons generating the single.

Without J-coupling there would be one methine proton peak and one methyl proton peak, but in reality these peaks are split as illustrated in Figure 1.3b. The frequency peak of a spin is split according to the spin orientation of the non-identical spin or spins it is coupled to. In the example of lactate (Figure 1.3b), the three identical methyl protons are split into two peaks of equal area because the spin of the single coupled methine proton is either parallel or up (low energy state) or antiparallel or down (high energy state) with the static magnetic field. The single methine proton is split into four peaks with relative areas in a 1:3:3:1 ratio because it is coupled to three methyl protons with four possible spin configurations: (up, up, up) one way, (up, up, down) three ways, (up, down, down) three ways, and (down, down, down) one way. In general, a J-coupled nucleus will be split into $n + 1$ peaks from $n$ magnetically equivalent nuclei, and the amplitudes of the split peaks are given by Pascal’s triangle (e.g. 1:1, 1:2:1, 1:3:3:1, etc.). The frequency difference between the split peaks is defined by the J-coupling.
constant in units of Hz, and is the same for each of the coupled nuclei. J-coupling is undesirable in MRS since the would-be single large peak is spread out into multiple smaller peaks, decreasing the SNR and creating complex overlapping within \textit{in vivo} spectra. Also, J-coupling can act as a source of transverse relaxation, further hindering the SNR of the J-coupled peaks.

**1.4.5 Multiple receiver signal combination**

A phased-array radiofrequency (RF) coil is comprised of N coil elements that independently detect the MR signal, resulting in N FIDs per acquisition. The FIDs can be combined as described by Brown et al \cite{86} using three properties of each coil element \textit{n}: a sensitivity weighting factor \( w_n \), a proximity weighting factor \( a_n \), and a phase factor \( \varphi_n \). The sensitivity weighting factors \( w_n \) are calculated as:

\[
W_n = \frac{\sigma_n}{\sqrt{\sigma_1^2 + \sigma_2^2 + \ldots + \sigma_n^2}}
\]  

(1.32)

where \( \sigma_n \) is the standard deviation of the noise in each element measured using the tail-end of each FID. The proximity weighting factors \( a_n \) are simply the maximum amplitudes of the FID from each coil element, normalized by the amplitude of the largest FID. The phase compensation factors \( \delta \varphi_n \) are calculated as:

\[
\delta \varphi_n = \varphi_n - \varphi_{\text{ref}}
\]  

(1.33)
where \( \varphi_n \) is the phase of the first complex data point of each FID and \( \varphi_{\text{ref}} \) is an arbitrarily chosen reference phase. The combined signal \( S(t) \):

\[
S(t) = \sum_{n}^{N} \left( w_n a_n e^{-\delta \varphi_n} S_n(t) \right)
\]

is given by the weighted sum of the coil element signals \( S_n(t) \).

### 1.5 MRS spectral quantification

#### 1.5.1 Lineshape corrections

The net transverse magnetization induces a sinusoidally varying current in the receiver coil at the Larmor frequency that decays exponentially due to transverse relaxation. Following Fourier transform this signal produces a Lorentzian lineshape in the frequency domain. However, lineshape distortions arise from magnetic field (\( B_0 \)) inhomogeneities and induced eddy currents (87,88) leading to non-Lorentzian lineshapes in the frequency-domain signal and errors in spectral peak area estimations when fitting with Lorentzian lineshapes (77,89,90). Two popular methods used to correct lineshape distortions are the QUALITY (QUAntification improvement by converting LIneshapes to the lorentzian TYpe) deconvolution (91) and eddy current correction (ECC) (88). QUALITY deconvolution consists of dividing the time-domain signal by a reference time-domain signal that has experienced the same \( B_0 \) inhomogeneities, typically the unsuppressed water signal, restoring Lorentzian lineshapes. However, the \( T_2 \) of water in the brain is shorter (~50 ms, (92)) than
the T₂ of most metabolites (~300 ms, (93)), leading to large signal spikes towards the end of the corrected time-domain signal because of division by near-zero water signal values. Eddy currents induced by magnetic field gradients produce time-dependent shifts of the resonance frequency, also resulting in distortion of the spectrum in the frequency domain (88). ECC is performed in the time domain by subtracting the phase of a reference signal (unsuppressed water) that has undergone the same distortions, from the phase of the signal to be corrected. However, ECC only corrects for B₀(t) distortions and therefore does not fully restore the Lorentzian lineshape. Bartha et. al. (94) combined QUALITY deconvolution and ECC (QUECC) to produce lineshape-corrected data without signal spikes by performing QUALITY deconvolution on the initial portion of the time-domain data followed by ECC on the remaining data points.

1.5.2 Echo time considerations

Most clinical studies use long echo time (TE > 100 ms) MRS to detect changes in N-acetylaspartate (NAA), total creatine (tCr), and choline (Cho = GPC + PC). Long-TE spectra (e.g. TE = 135 ms, 270 ms) are relatively simple to analyze as they contain easily resolved peaks from NAA, tCr, and Cho and few overlapping signals from macromolecules (95). In contrast, acquisition with short-TE (TE < 50 ms) produces visible peaks from additional metabolites including glutamate (Glu), glutamine (Gln), and myo-inositol (mI) as well as broad peaks from macromolecules. Metabolite quantification then becomes more difficult due to the overlapping macromolecule resonances that extend beneath the 2-4 ppm metabolite region and overlap with several metabolites of interest (e.g. Glu, Gln, and tCr).
Short-TE MRS is able to detect more metabolite signals for two reasons. First, the signals from species with short $T_2$ relaxation times have not appreciably decayed, and second, signal loss from J-coupling phase modulation is reduced.

### 1.5.3 Macromolecule removal

One approach to account for the macromolecule contribution is to include prior information about the macromolecule lineshapes in the fitting (79). However, macromolecule resonances have been shown to fluctuate in disease conditions (96), potentially leading to inaccurate metabolite measurements (97).

Alternatively, a subject-specific macromolecule spectrum can be acquired in addition to the metabolite spectrum and subsequently used to subtract the macromolecule contributions from the metabolite spectrum (97). Although this approach increases acquisition time, it provides the most direct method to identify subject specific macromolecule contributions. A macromolecule spectrum, free of metabolite signals, can be directly measured by inversion-nulling the metabolite signals (98,99). Inversion-nulling refers to applying a 180° pulse followed by a specific time delay (TI), defined by the $T_1$ of the signal to be nulled, before the 90° excitation pulse rotates the magnetization into the transverse plane for detection. The longitudinal magnetization following a 180° pulse is:

$$M_z(t) = M_0 \left(1 - 2e^{-\frac{t}{T_1}}\right)$$

(1.35)
obtained by setting $M_z(0) = -M_0$ and substituting into Equation 1.23. At 7T the longitudinal relaxation times ($T_1 = 1/R_1$) of macromolecules ($T_1 \sim 430$ ms) are shorter than metabolites ($T_1 \sim 1600$ ms) (100,101). Therefore the magnetization from the macromolecule protons relaxes back along the longitudinal axis more quickly than that from the metabolite protons, as illustrated in Figure 1.4.

![Figure 1.4: Longitudinal magnetization for macromolecules and metabolites following a 180° inversion pulse. The $T_1$s of macromolecules are shorter (~350 ms at 7T) than metabolites (~1200 ms at 7T), and therefore the macromolecule magnetization realigns with the longitudinal axis much more quickly than the metabolite magnetization. Applying a 90° excitation pulse at $t_{null}$ will rotate magnetization from the macromolecules into the transverse plane, allowing the direct measurement of the macromolecule signals without overlapping metabolite signals.](image-url)
Setting the delay time (TI) equal to the time where the magnetization from the metabolite protons crosses zero (t\text{null}) will result in no excitation of the metabolite signals when the 90° pulse is applied, in theory producing a pure macromolecule spectrum. The macromolecule spectrum can then be subtracted from the full spectrum (containing metabolite and macromolecule signals) to produce a macromolecule-free metabolite spectrum as illustrated in Figure 1.5. One advantage of macromolecule measurement and subtraction is that the resulting metabolite only spectrum will be void of complicating macromolecule resonances. An advantage for acquiring a macromolecule spectrum alongside each full spectrum, as opposed to using a generalized macromolecule baseline function in the fitting, is that macromolecule removal will be more accurate in cases where the macromolecule baseline fluctuates. Disadvantages of this approach to remove macromolecule resonances include: a nearly two-fold increase in MRS scan time, and the macromolecule subtraction introduces some error in the resulting metabolite spectrum peaks.
Figure 1.5: The full spectrum containing both metabolite and macromolecule signals, the macromolecule spectrum with metabolites suppressed, and the resulting metabolite spectrum following macromolecule subtraction.

1.5.4 Fitting to metabolite prior knowledge

The area under a peak in a MR spectrum is directly proportional to the number of nuclei resonating at that particular frequency, and therefore also proportional to the number of molecules in which the nuclei originate. As a result, the concentration of a molecule can be determined from the area of the associated resonance peak or peaks. Integration over the
width of a peak in the frequency domain is the simplest approach to determine the area of a peak, but this approach not practical for in vivo short echo-time $^1$H MRS data due to the overlapping of metabolite peaks. Further, although the time and frequency domain representations contain identical information due to the linearity of the Fourier transform (FT), in practice the frequency domain data may have severe artifacts such as baseline distortions caused by truncation of the FID in the time domain. Therefore, quantitative analyses of metabolite spectra are often performed in the time domain to avoid any distortions induced by the FT of the data. One approach to the analysis of short echo-time $^1$H MRS data involves the use of metabolite lineshapes. The incorporation of such prior knowledge obtained from in vitro metabolite solutions greatly simplifies spectral fitting. Spectra are reconstructed using linear combinations of known metabolite lineshapes to determine the contribution of each to the in vivo spectrum. Figure 1.6 shows a typical in vivo $^1$H MR spectrum acquired from healthy human brain at 7T, along with the fitted contributions from the major metabolites of interest.
Figure 1.6: A typical 7T in vivo $^1$H MR spectrum from healthy human brain with superimposed fit in orange (a), residual difference between the data and fit (b), and individual metabolite components from NAA (c), Glu (d), Gln (e), tCr (f), Cho (g), and mI (h).

The lineshape of each metabolite to be included in the in vivo fitting template can be obtained from fitting a high-resolution in vitro spectrum (77,91) acquired from an aqueous solution of that respective metabolite, described in detail by Bartha et. al. (89). Sodium 3-
trimethylsilyl-propionic acid (TSP) is used as a chemical shift and Lorentzian damping reference (89) for each metabolite lineshape. This metabolite fitting method has been successfully demonstrated by several groups at field strengths ranging from 1.5T to 9.4T (79,96-99,101-104), however it is only accurate when all contributions to the in vivo spectrum are incorporated during fitting (101).

Prior knowledge metabolite templates can be fitted to MRS data by minimizing the difference between the reconstructed spectrum and the data using a Levenberg-Marquardt algorithm for nonlinear least squares optimization (105). The nth point of the reconstructed FID is given by (106):

$$x_n = \sum_{m} A_m e^{i(\phi_0 + \phi_{1,m})} e^{-(R_{2,m} + i\Delta\omega_m)t_n}$$

(1.36)

where M is the total number of sinusoids. Equation 1.36 describes each resonance (m, peak in the spectrum) with the following parameters: amplitude ($A_m$), frequency-independent zero-order phase ($\phi_0$), frequency-dependent first-order phase ($\phi_{1,m}$), transverse decay rate ($R_{2,m} = 1/T_{2,m}$), and chemical shift ($\Delta\omega_m = \omega_m - \omega_0$). In the fitting template each resonance’s chemical shift and Lorentzian damping are linked to common parameters using the TSP reference, and relative amplitudes of different resonances from the same metabolite are linked to each other. Linking these parameters both simplifies the fitting by reducing the number of parameters to vary and ensures proper classification of each resonance as the
chemical shifts and relative amplitudes are fixed. Only the relative amplitudes from difference metabolites are allowed to vary.

### 1.5.5 Signal relaxation corrections

Accurate quantification of metabolite levels from *in vivo* spectra requires amplitude corrections that incorporate the different relaxation times ($T_1$ and $T_2$) of water and metabolite protons. For instance, during the time between excitation and the formation of the spin echo (TE), signals from water protons decay more than signals from metabolite protons because of their shorter $T_2$s, and these differences must be accounted for when attempting to measure absolute metabolite levels. Similarly, differences in $T_1$ values between water and metabolites result in different amounts of longitudinal relaxation between consecutive MRS scans, and must be accounted for before absolute quantification. Further complicating relaxation correction is the fact that relaxation times vary with tissue type. As an example, water at 4T has $T_1$ values of 1348 ms and 904 ms and $T_2$ values of 70 ms and 55 ms in GM and WM, respectively (106). Errors in metabolite level measurement may arise when incorporating relaxation correction due to the uncertainty in $T_1$ and $T_2$ time constants. These values are often difficult to measure for all metabolites, and additionally, pathological conditions can alter the normal $T_1$ and $T_2$ values. The use of pulse sequences with short echo-times and long repetition times minimizes these errors caused by these uncertainties.
1.5.6 Partial volume correction

The water content within the MRS measurement volume can be used as an internal reference (107,108) as the concentration of water is well known within brain tissue (77,107,108). However, the accurate quantification of metabolite concentrations then requires measurement of tissue partial volume within the voxel as each tissue type has a specific water concentration. One approach to determine the proportions of cerebral spinal fluid (CSF), grey matter (GM), and white matter (WM) is to segment (109,110) high resolution anatomical images acquired during the same imaging session. The proportions of each tissue type, along with their respective water concentrations, are then used to assign the correct concentration to the reference water signal.

Another approach to estimate voxel CSF content is to collect a series of water spectra at a range of echo times to measure the water signal transverse decay. This approach can separate the water decay data into CSF ($T_2 = 141\text{ ms at 7T (92)}$) and tissue (GM + WM) components (at 7T, $T_{2GM} = 50\text{ ms, } T_{2WM} = 55\text{ ms (92)}$), to give the proportions of CSF and tissue water within the voxel (Figure 1.7). Since the $T_2$ values of GM and WM are so close, this method cannot reliably separate the components of GM and WM and therefore they are combined into the tissue component. The ideal approach would be to correct for signal relaxation effects by separating GM and WM components because of their differences in $T_1$ and $T_2$ values. However, this method yields accurate results because the signal attenuation corrections for GM and WM (using TR, TE, $T_1$, and $T_2$ values) do not differ substantially (<5% at 7T with TR/TE = 3700/38 ms).
Figure 1.7: Representative plot of normalized water signal versus echo time (TE), with superimposed two-component exponential decay fit. The individual contributions from CSF and tissue are also shown, illustrating their initial water signal amplitudes and decay rates.

This separation is accomplished by acquiring water spectra at a range of TEs and then fitting the data to a two-component exponential decay equation:

\[ S(TE) = S_0 + A_{\text{CSF}} \exp\left(-\frac{TE}{T_{2,\text{CSF}}}\right) + A_{\text{tissue}} \exp\left(-\frac{TE}{T_{2,\text{tissue}}}\right) \]  

(1.37)

where \( A_{\text{CSF}} \) and \( A_{\text{tissue}} \) are the initial amplitudes of the CSF and tissue signals, respectively. In the example shown in Figure 1.7 there is more tissue in the MRS measurement volume than
CSF. In this case, the tissue water signal is initially large but decays more quickly due to its shorter $T_2$ (~50 ms) than CSF (~150 ms) (92).

1.6 Thesis overview

This introductory chapter provided an overview of the neuropathology, current diagnosis, and current treatment of Alzheimer disease. Secondly, current neuroimaging methods used to study AD were presented, along with a detailed description of the major metabolic changes as measured by proton magnetic resonance spectroscopy ($^1$H MRS). Lastly, pertinent principles of magnetic resonance (MR), MR imaging, and MR spectroscopy were presented, with a detailed description of the steps involved in MRS data acquisition, post-processing, and quantification.

Chapter 2 presents a short echo time LASER $^1$H MRS study at 4T where metabolite concentrations were measured in subjects with AD following four months of galantamine treatment. Chapter 3 presents a long echo time PRESS $^1$H MRS study at 3T where metabolite concentrations were measured in subjects with AD following four months of rivastigmine treatment. Chapter 4 presents the details of a short echo-time semi-LASER $^1$H MRS data acquisition and quantification protocol at 7T, incorporates subject-specific macromolecule removal, to determine absolute metabolite levels in the human brain. Chapter 5 will provide a summary of this thesis, and will describe future work and limitations of this research.
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Chapter 2

Increased glutamate in the hippocampus after galantamine treatment for Alzheimer disease

2.1 Introduction

Alzheimer Disease (AD) is the most common form of dementia and it is a progressive, degenerative disease of the brain resulting in cognitive and memory impairments. The clinical progression of AD is currently measured by cognitive assessments, most commonly using the Mini-Mental State Examination (MMSE) (1) or the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) (2,3). However, these cognitive assessments offer modest sensitivity at best (2,4,5), particularly in early stages of the disease. In an effort to improve monitoring of disease progression and response to treatment, various non-invasive imaging methods are under development.

One such biomarker for disease status is proton magnetic resonance spectroscopy (\(^1\)H MRS), which can be used to measure several metabolites in the human brain. Although the neurochemical abnormalities of AD assessed by \(^1\)H MRS are not completely defined, there is evidence of progressive chemical changes that involve multiple brain regions. These changes include decreased N-acetylaspartate (NAA) or NAA/total creatine (tCr = Cr + PCr) in the parietal and occipital cortex (6), gray matter (7,8), hippocampus (9-11), and posterior cingulate (12) as well as increased myo-inositol (mI) in parietal and occipital cortex (6), gray matter (7), and posterior cingulate (12). Since NAA is a marker of neuronal integrity or viability, these studies consistently demonstrate loss of neuronal function in subjects with AD. The increase in mI is associated with gliosis (13). The tCr concentration is typically used as a stable relative control value.

Previous studies performed at magnetic field strengths of \(\leq\) 3 Tesla (T) have also noted changes in glutamate (Glu) + glutamine (Gln) concentration, commonly reported as Glx. Glu
is a major excitatory neurotransmitter that has been shown to decrease with age in control subjects (14), while Gln is a precursor of Glu. Glu excitotoxicity is a feature of AD (15), therefore it is becoming increasingly important to understand how this metabolite could be used as a marker of disease progression and response to therapy. Previous studies have reported decreased Glx or Glx/tCr ratio in subjects with AD compared to control subjects in occipital gray matter (7), in the cingulate cortex (16), and in the posterior cingulate gyrus and precuneus (17). Decreased Glx levels have also been reported over approximately 12 months in the posterior cingulate gyrus in subjects with mild cognitive impairment (MCI) (18).

$^1$H MRS has successfully been used to evaluate AD progression (11,19) and response to treatment (20-22). However $^1$H MRS treatment response studies have been limited to donepezil (20,21) and rivastigmine (22), two acetylcholinesterase inhibitors (AChEI) in clinical use that have been shown to provide symptomatic relief for mild to moderate AD (23) and increase glucose metabolism (24). A third approved treatment, galantamine (Gal), has yet to be studied by $^1$H MRS. Gal inhibits acetylcholinesterase (AChE) activity, but also acts as an allosteric potentiating ligand for presynaptic nicotinic acetylcholine receptors (nAChR) (25). In the hippocampus, this modulatory function may facilitate glutamatergic neurotransmission resulting in beneficial effects on learning and memory. This increase in glutamatergic transmission may lead to increases in intracellular Glu levels detectable by $^1$H MRS. In addition, Gal may prevent Glu neurotoxicity leading to neuronal apoptosis (26). Therefore it is of particular interest to determine whether Gal modulates Glu levels following treatment.
As technology improves and the use of high magnetic field strength (≥ 3 T) magnetic resonance imaging (MRI) becomes more accessible, 1H MRS spectra can be acquired with a greater signal-to-noise ratio and improved spectral resolution (3,27,28), generally improving overall metabolite quantification. We have previously shown that the use of short echo-time 1H MRS at 4 T in combination with advanced metabolite quantification methods can be used to measure Glu levels in the hippocampus (20,29). The hippocampus is of particular interest as it is known to be involved early in the pathogenesis of AD. In a recent study using 4T 1H MRS, we found that glutamate levels were decreased in the right hippocampus in subjects with AD compared to normal elderly controls (30). Based on this result and the mechanism of action of Gal, we hypothesized that 4 months of Gal treatment would result in detectable changes in the 1H MRS metabolite profile measured from within the right hippocampus of patients with AD, specifically that levels of Glu would increase due to increased glutamatergic neurotransmission. The right hippocampus was chosen to allow a direct comparison with a previous study by our group examining metabolite level changes following donepezil treatment (20). Therefore, the purpose of this study was to measure the metabolite level changes within the right hippocampus in response to four months of Gal treatment using 1H MRS, and to correlate metabolic changes with standard clinical outcomes (MMSE and ADAS-cog scores).
2.2 Methods

2.2.1 Patient Population

Subjects who met the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA) criteria for probable AD were recruited from the Aging Brain and Memory Clinic in London, Ontario, Canada. Ten subjects participated in this study, which was approved by the University of Western Ontario Health Sciences Research Ethics Board. The MMSE and the ADAS-cog were performed on the participants immediately before (baseline) and four months after beginning Gal treatment. After the baseline MRI scan, subjects were started on galantamine ER 8 mg/day after breakfast for the first month, and then increased to 16 mg/day for the remainder of the study. Subjects were maintained on this dose following the post dose MRI scan at 4 months. This two-step dosing approach mirrored the same two-step approach previously used to study the effects of donepezil (Bartha et al., 2008). Although a maximum dose of 24 mg/day is possible with galantamine it may be less well tolerated than 16 mg/day. Therefore, a maximum dose of 16 mg/day was chosen to avoid losing subjects for the post-dose MRI component of this study. Additionally, there is no randomized controlled trial data suggesting any statistically significant greater clinical benefit for 24 mg/day compared to 16 mg/day. It was also considered that most older people have reduced renal function, i.e. creatinine clearance of <60 mL/min. ¹H MRS was used to measure the concentrations of metabolites within the right hippocampus of the participants at baseline and at 4 months. The study participant demographics are summarized in Table 2.1.
Table 2.1: Demographics of study participants

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 months</th>
<th>p-value#</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>10</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td>7F/3M</td>
<td>7F/3M</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>81.5 ± 6.0</td>
<td>81.9 ± 6.0</td>
<td>-</td>
</tr>
<tr>
<td>MMSE</td>
<td>25.9 ± 2.0</td>
<td>24.7 ± 1.9 (N = 9)</td>
<td>0.10 (N = 9)</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>15.2 ± 4.2</td>
<td>15.1 ± 6.5 (N = 9)</td>
<td>0.82 (N = 9)</td>
</tr>
<tr>
<td>Education (years)</td>
<td>12.9 ± 3.8</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

N - number of participants, F - female, M - male
Age, MMSE, ADAS-cog, and Education values listed as average ± standard deviation.
# - p-values for two-tailed repeated measures t-tests

2.2.2 Assessment Instruments

All images and $^1$H MR spectra were acquired using a Varian (Palo Alto, CA) whole body 4 T MRI scanner with a Siemens (Erlangen, Germany) Sonata gradient coil. At the start of each study, a series of images were acquired parallel to the hippocampus (Figure 2.1) using a 3D inversion prepared T$_1$-weighted Fast Low Angle Shot (FLASH) imaging sequence (TI/TR/TE = 500/9.5/5 ms, 256x256 slice resolution, 16 slices, 2.5 mm thickness, FOV = 24 cm) for placement of the $^1$H MRS voxels. $^1$H MRS data were acquired as previously described (29,30) from a voxel ranging in volume from 2.8 cm$^3$ to 4.7 cm$^3$ (average ± standard deviation = 3.5 ± 0.6 cm$^3$) positioned within the right hippocampus (Figure 2.1).
Figure 2.1: Transverse oblique $T_1$-weighted FLASH image (FOV = 22 cm, slice thickness = 2 mm, TI/TR/TE = 500/9.5/5 ms) of a patient with Alzheimer disease acquired parallel to the hippocampus on a Varian/Siemens 4T MRI system. The spectroscopy voxel outlined in white is positioned within the right hippocampus.

The spectroscopy acquisition utilized variable pulse power and optimized relaxation delays (VAPOR) water suppression (31) followed by short echo-time (TE/TR = 46/3200 ms) localization by adiabatic selective refocusing (LASER, 2 kHz receiver bandwidth, $\tau_{cp} = 6$ ms) (32). For each scan, a full spectrum (containing both the metabolite and macromolecule signals), a macromolecule spectrum (metabolite suppressed macromolecule signal), and a water spectrum (containing the unsuppressed water signal) were acquired (29). Metabolite
suppression was achieved by two-pulse inversion nulling (33), using adiabatic full passage (HS2-R10) pulses (TI\textsubscript{1} = 2.20 s, TI\textsubscript{2} = 0.69 s). The resulting spectra were lineshape corrected (34) to eliminate eddy current distortions. The macromolecule spectrum was fit using an automated Hankel singular value decomposition (HSVD) fitting routine (29,35) and scaled by 1.2 to account for T\textsubscript{1}-saturation before subtracting it from the full spectrum. Any remaining unsuppressed water signal was then removed by subtracting resonances between 4.1 and 5.1 parts per million (ppm) (water ~ 4.7 ppm) as determined by the HSVD algorithm (29).

2.2.3 Data analysis

Metabolite levels were quantified by fitting the resultant metabolite spectra in the time domain (fitMAN software (36)) using a Levenberg-Marquardt minimization routine (37) incorporating prior knowledge from 19 metabolite lineshapes as previously described (29,30,36). Metabolite levels were normalized to the unsuppressed water level and then corrected for T\textsubscript{1} and T\textsubscript{2} relaxation effects (29,30,38). The water content was estimated to be 81% in the hippocampus and 71% in the white matter (39,40). Table 2.1 in Kassem and Bartha (29) lists the T\textsubscript{1} and T\textsubscript{2} values for water and the metabolites of interest in gray matter and white matter of young healthy subjects at 4 T. These same values were used in the current study for subjects with AD except the T\textsubscript{2} of water was reduced by 15% to 86 ms based on the results of Haley et al (41) in subjects with AD. No adjustment was made for the T\textsubscript{2} of the metabolites as it has been shown that the T\textsubscript{2} of several metabolites are the same for young controls and subjects with AD (7).
Measured metabolite concentrations were also corrected for the amount of cerebral spinal fluid (CSF) contained in the measurement voxel. The relative amounts of gray matter, white matter and CSF in each voxel were determined by segmenting the T1-weighted 3D FLASH images (Figure 2.1) using statistical parametric mapping (14) in a program called SPM5 (42,43). To achieve consistent segmentation each image was transformed onto a template using a rigid body transformation before the segmentation was performed and a template of tissue probability was utilized. The contribution of gray matter, white matter, and CSF to each voxel in normal tissue was calculated using a plug-in written in ImageJ (National Institute of Health).

The metabolites NAA, Glu, tCr, choline (Cho = GPC + PC), and mI were included in statistical comparisons based on previous studies that have implicated these metabolites in AD pathophysiology, and because these metabolites could be reliably measured. Paired two-tailed repeated measures t-tests were used to compare baseline and 4 month absolute metabolite concentrations. In a secondary analysis the ratios of NAA/tCr, Glu/tCr, Glu/NAA, and Glu/mI were also compared, as were the MMSE and ADAS-cog scores. A Bonferroni correction was applied to the comparison of absolute metabolite levels and metabolite ratios. In the case of absolute metabolite levels, the corrected $p$-value for significance was $p < 0.01$ (5 comparisons made), while for the metabolite ratios the corrected $p$-value for significance was $p < 0.0125$ (4 comparisons). $P < 0.05$ was used for comparison of cognitive scores. Linear regression analysis was performed comparing the change in metabolite levels with the change in cognitive scores.
2.3 Results

Figure 2.1 shows a typical image used in positioning the MRS voxel, shown as a gray box, within the right hippocampus. Figure 2.2 shows the spectrum acquired from one subject at baseline along with the fitted result and the residual (the difference between the fit and the spectrum).

Figure 2.2: The individual spectra representing NAA (A), Glu (B), tCr (C), Cho (D), and mI (E) are shown beneath the acquired magnetic resonance spectrum (in gray) and the fitted result (black, F). The residual (G) is the difference between the acquired spectrum and the fit result.
The individual metabolite components for NAA, Glu, tCr, Cho, and mI that contributed to the spectrum are also provided in Figure 2.2. In the voxels studied, the average proportions of gray matter, white matter, and cerebral spinal fluid were 54%, 27%, and 19% respectively at baseline and 54%, 26%, and 20% respectively at 4 months.

The average absolute metabolite levels at baseline and 4 months are shown in Figure 2.3. Glu increased by 35% (p < 0.01), while NAA, tCr, Cho, and mI did not change significantly.

![Figure 2.3: Average absolute metabolite concentrations at baseline (BL) and after 4 months (4m) of galantamine treatment in the same patients. The errors bars represent the standard deviation, and the asterisk represents a significant difference between baseline and 4 months (p < 0.01). The average metabolite ratios at baseline and 4 months are shown in Figure 2.4. The ratio of]
Glu/tCr increased by 46% (p < 0.01) and Glu/NAA increased by 54% (p < 0.001).

Figure 2.4: Average metabolite ratios at baseline (BL) and after 4 months (4m) of galantamine treatment in the same patients. The error bars represent the standard deviation, and single and double asterisks represent significant differences between baseline and 4 months with p < 0.01 and p < 0.001, respectively.

Interestingly, the change (Δ) in Glu (ΔGlu) correlated with the ΔNAA (Figure 2.5, p < 0.05), although NAA did not change significantly.
The association between the change in $N$-acetylaspartate and the change in glutamate. The black line represents the linear fit to the data.

The average MMSE and ADAS-cog scores at baseline and 4 months are provided in Table 2.1. The 4 month cognitive scores of one subject were excluded from the analysis as this individual had an uncharacteristic lack of interest during this testing session resulting in poor scores that improved a few months after treatment with donepezil and venlafaxine. Although the 4 month cognitive scores from this subject were excluded from the analysis, the subject’s MRS data was included in the analysis as the MRS levels are the primary measure of this study and would not likely be effected by the lack of interest of an individual. The MMSE and ADAS-cog scores and the ratio of Glu/mI showed no change between baseline and 4 months, however the change in the ratio of Glu/tCr ($\Delta$(Glu/tCr)) was correlated with
the change in the MMSE scores (ΔMMSE) (Figure 2.6, p < 0.05), and the change in the ratio of Glu/ml (Δ(Glu/ml)) was inversely correlated with the change in the ADAS-cog scores (Figure 2.7, p < 0.05).

Figure 2.6: The association between the change in the ratio of Glu/tCr and the change in MMSE scores. The black line represents the linear fit to the data.
2.4 Discussion

The purpose of this study was to measure metabolite level changes within the right hippocampus of newly diagnosed subjects with AD in response to four months of Gal treatment using $^1$H MRS, and determine if these changes correlated with changes in cognitive function measured using MMSE or ADAS-cog scores. Absolute Glu levels were found to increase in subjects with AD following 4 months of Gal treatment, as did the ratios of Glu/tCr and Glu/NAA.

Although several studies have shown changes in metabolite concentrations in AD, few studies have examined treatment response (summarized in Table 2.2).
Table 2.2: Summary of previously reported treatment effects

<table>
<thead>
<tr>
<th>Study</th>
<th>Treatment</th>
<th>Interval</th>
<th>Location</th>
<th>Findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bartha et al, 2008</td>
<td>Don</td>
<td>16 weeks</td>
<td>Right hippocampus</td>
<td>Decreased NAA, NAA/tCr, &amp; ml/tCr compared to baseline</td>
</tr>
<tr>
<td>Jessen et al, 2006</td>
<td>Don</td>
<td>12 weeks</td>
<td>Parietal lobe</td>
<td>Correlation of ΔNAA and ΔNAA/tCr with ΔADAS-cog</td>
</tr>
<tr>
<td>Krishnan et al, 2003</td>
<td>Don</td>
<td>12 &amp; 18 weeks</td>
<td>Periventricular white matter</td>
<td>Increased NAA compared to control</td>
</tr>
<tr>
<td></td>
<td></td>
<td>12 weeks</td>
<td>Subcortical gray matter</td>
<td>Increased NAA compared to control</td>
</tr>
<tr>
<td>Modrego et al, 2006</td>
<td>Riv</td>
<td>16 weeks</td>
<td>Frontal cortex</td>
<td>Increased NAA/tCr compared to baseline</td>
</tr>
<tr>
<td></td>
<td></td>
<td>16 weeks</td>
<td>Occipital cortex</td>
<td>Increased ml/tCr compared to baseline</td>
</tr>
</tbody>
</table>

Don - donepezil, Riv - rivastigmine

Decreased NAA or NAA/tCr and increased ml have been reported most consistently in subjects with AD that are not receiving treatment (6-12). Of the few studies that have examined treatment effects, one study found a correlation between changes in NAA and NAA/tCr with changes in ADAS-cog in the parietal lobe after 12 weeks of donepezil treatment (44). A second study found a higher concentration of NAA in subjects who received donepezil treatment than those who received placebo at 12 and 18 weeks in the periventricular matter and at 12 weeks in the subcortical gray matter (21). A previous study by our group using the same methodology as the current study showed decreased NAA and NAA/tCr levels as well as ml/tCr levels, and no change in Glu levels in the right hippocampus after 4 months of donepezil treatment (20). These metabolic changes indicate
continued neuronal impairment following treatment, although the change in mI/tCr was attributed to an effect of the medication since mI is normally increased in AD. A fourth study showed increased NAA/tCr in the frontal cortex and increased mI/tCr in the occipital cortex after 4 months of rivastigmine treatment (22). The variability in these previous studies may indicate a differential effect of brain region. The current study is the first to report metabolite changes associated with Gal treatment and did not show an alteration in either NAA levels, mI/tCr levels, or average cognitive scores over four months.

Gal is expected to exert effects on the glutamatergic system. However, the measurement of tissue Glu levels by $^1$H MRS requires the use of short-echo time acquisitions and advanced quantification methods (29,30,35). The use of magnetic field strengths of < 3 T also makes it difficult to separate overlapping resonances in the spectrum from Glu and Gln, resulting in the common reporting of a combined Glu plus Gln measure called Glx. A few previous studies have reported decreased Glx in occipital cortex (7), the cingulate cortex (16), the gray matter of the posterior cingulated gyrus and precuneous (17), and no change in the midfrontal and temperoparietal gray matter (45) in subjects with AD. Recently, we have also shown decreased Glu in the right hippocampus in subjects with AD (30). These studies are supported by a study in a non-treated transgenic AD mouse model that showed a decrease in Glu in progressive AD (46). One might expect decreased Glu along with decreased NAA as they both exist mainly in neuronal pools and neuronal degradation is consistently reported in progressive AD. However, the finding of the current study indicating increased Glu following Gal treatment suggests that measured Glu levels may be related to neurotransmission and cognitive function. A post-hoc analysis also found no differences in
glutamine levels (data not shown) in the current study following treatment, providing further support for the observed changes in glutamate (since glutamate and glutamine resonances overlap in the $^1$H spectrum). The observed positive correlation between the change in Glu/tCr and the change in MMSE score, and the inverse correlation between the change in Glu/mI and the change in ADAS-cog score suggest that these metabolite ratios are related to cognitive function and performance.

It has been reported that Gal improves cognitive functions in subjects with AD by increasing the activity of the cholinergic system (23). Neurotransmission within the hippocampus involves the interaction of both glutamatergic and cholinergic signal transduction mechanisms. AChEIs reduce the breakdown of acetylcholine thereby increasing cholinergic neurotransmission. Gal is unique among AChEIs approved for treatment of AD as it is a weak cholinesterase inhibitor, but is efficiently able to allosterically stimulate α7 nAChRs (47-49). The α7 nAChR is an ion channel found in high concentration in the hippocampus that allows entry of $^{2+}$Ca into the cell (50,51). Gal binds to nAChRs and acts as an allosteric potentiating ligand, sensitizing the receptor to activation by acetylcholine, which in turn stimulates glutamatergic release (48). The increase in Glu release stimulates post-synaptic N-methyl-D-aspartic acid (NMDA) receptors and produces beneficial effects on learning and memory. Thus the increase in Glu observed in the hippocampus in the current study may be related to the action of Gal as an allosteric potentiating ligand for α7 nAChRs. Activation of α7 nAChRs by Gal may also prevent Glu neurotoxicity (52-54) and the subsequent apoptosis of neurons containing Glu, which could also result in a higher Glu concentration.
Although this study includes a small sample size of ten subjects, the statistical power associated with the change in absolute Glu concentration is 0.94. Further limitations of this study include only measuring the right hippocampus over a short follow-up time of 4 months. Although the short follow-up time may be considered an advantage from the point-of-view of the metabolite measurements, it may contribute to learning effects associated with the cognitive assessment. Such a learning effect may contribute to a false positive response. However, there is sufficient evidence in the cholinesterase inhibitor literature when using the ADAS-cog as a measure of cognition in patients with dementia to justify reliable results within a timeframe of 4 months (55-57). Nonetheless, the significant increase in absolute Glu indicates that Glu levels may be an important metabolite marker in assessing AD progression and response to treatment. Although further studies are needed, non-invasive metabolite measurements could be used in conjunction with clinical markers of cognition, function, and behavior, to verify treatment response.

2.5 Conclusions

Short echo time $^1$H magnetic resonance spectroscopy at 4 Tesla was used to measure metabolite concentrations within the right hippocampus of 10 subjects with AD following four months of galantamine treatment. The absolute Glu concentration increased, as did the ratios of Glu/tCr and Glu/NAA. The change in the ratio of Glu/tCr was positively correlated with the change in the MMSE scores and the change in the ratio of Glu/ml was inversely correlated with the change in the ADAS-cog scores, both suggesting that these metabolite ratios are related to cognitive function. Further studies are required to determine whether the
increase in Glu continues with galantamine treatment during further disease progression.
2.6 References


Chapter 3

Reduced N-acetylaspartate to creatine ratio in the posterior cingulate correlates with cognition in Alzheimer disease following four months of rivastigmine treatment

A version of this chapter has been accepted for publication. Penner J, Wells JL, Borrie MJ, Woolmore-Goodwin SM, Bartha R. Reduced N-acetylaspartate to creatine ratio in the posterior cingulate correlates with cognition in Alzheimer disease following four months of rivastigmine treatment. Dement Geriatr Cogn Disord. Accepted August 19, 2014.
3.1 Introduction

Alzheimer disease (AD) is a neurodegenerative disease characterized by a progressive loss in memory, language, executive, and visuospatial functions. Cognitive assessments are predominantly used to monitor the clinical progression of AD (1,2). These assessments offer modest sensitivity to AD (2-4), but lack specificity (2-4). Brain imaging has also been intensively studied in the context of Alzheimer’s disease in an effort to find a biomarker that will improve the evaluation of disease progression. Methods include structural magnetic resonance imaging (MRI) (5-9), functional MRI (fMRI) (10-12), fluorodeoxyglucose positron emission tomography (FDG-PET) (13-15), amyloid PET (16-18), and cerebral blood flow single photon emission computed tomography (CBF-SPECT) (19). However, objective and rapid evaluation of treatment response using non-invasive imaging requires further development to direct treatment decisions that could postpone decline in cognitive performance.

The standard of care for patients with AD is treatment with a cholinesterase inhibitor (ChEI) (20,21). The ChEIs currently in clinical use are rivastigmine, donepezil, and galantamine. ChEIs have been developed based on the cholinergic hypothesis (22), which proposes that AD involves reduced synthesis of acetylcholine, which leads to reduced cholinergic neurotransmission from neurons originating in the basal forebrain (23,24). Treatment with rivastigmine produces positive effects on cognitive performance and activities of daily living in patients with AD (25-28). In a meta-analysis of all three second-generation ChEIs (27), donepezil and galantamine showed similar cognitive efficacy to rivastigmine, but rivastigmine is the only ChEI that is available as a transdermal patch.
Previous studies have reported that transdermal administration reduces gastrointestinal adverse events (29), provides sustained therapeutic drug plasma levels (30), and is preferred over capsules by 70% of caregivers due to ease of use (31).

To optimize patient care, rapid assessment of treatment response is needed. Although not considered disease modifying, ChEIs can induce metabolic changes in the brain measurable by proton magnetic resonance spectroscopy (1H MRS) (32-38). 1H MRS is a non-invasive imaging technique capable of directly detecting mobile metabolites in the human brain with concentrations greater than approximately 0.5 mM (39). There is mounting evidence of neurochemical abnormalities, assessed by 1H MRS, in multiple brain regions in AD (40-46). 1H MRS studies of treatment response have investigated donepezil (32,35), rivastigmine (37), and galantamine (38). Metabolite level changes have been reported in multiple brain regions following ChEI treatment (summarized in Table 3.1). However, only one study to date has measured the effects of rivastigmine, which was performed at 1.5 Tesla and examined the frontal, parietal, and occipital cortices (37). This previous study found increased NAA/tCr in the frontal cortex that correlated with ADAS-cog scores, increased mI/tCr in the occipital cortex, and no changes in cognitive scores after four months of rivastigmine treatment (37).
Table 3.1: Summary of previously reported ChEI treatment effects

<table>
<thead>
<tr>
<th>Study</th>
<th>ChEI</th>
<th>N</th>
<th>Int. (w)</th>
<th>Location</th>
<th>Findings</th>
<th>Interpolated ΔNAA/tCr over 4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Henigsberg et al., 2011</td>
<td>Don</td>
<td>12</td>
<td>26</td>
<td>Dorsolateral prefrontal cortex</td>
<td>Increased NAA/tCr vs baseline</td>
<td>+4.0%</td>
</tr>
<tr>
<td>Modrego et al., 2010</td>
<td>Don</td>
<td>32</td>
<td>26</td>
<td>Bilateral PC</td>
<td>Increased NAA/tCr vs baseline</td>
<td>+1.3%</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Left occipital</td>
<td>Increased mI/tCr vs baseline</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Right prefrontal</td>
<td>Increased mI/tCr vs baseline</td>
<td>-1.8%</td>
</tr>
<tr>
<td></td>
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<td></td>
<td></td>
<td>Left prefrontal</td>
<td>Decreased NAA/tCr vs baseline</td>
<td></td>
</tr>
<tr>
<td>Penner et al., 2010</td>
<td>Gal</td>
<td>10</td>
<td>16</td>
<td>Right hippoc</td>
<td>No change in NAA/tCr</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Increased Glu &amp; Glu/tCr vs baseline</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ΔGlu/tCr correlated with ΔMMSE</td>
<td></td>
</tr>
<tr>
<td>Bartha et al., 2008</td>
<td>Don</td>
<td>10</td>
<td>16</td>
<td>Right hippoc</td>
<td>Decreased NAA, NAA/tCr, &amp; mI/tCr vs baseline</td>
<td>-16%</td>
</tr>
<tr>
<td>Jessen et al., 2006</td>
<td>Don</td>
<td>17</td>
<td>12</td>
<td>Parietal lobe</td>
<td>No change in NAA/tCr</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>ΔNAA &amp; ΔNAA/tCr correlated with ΔADAS-cog</td>
<td></td>
</tr>
<tr>
<td>Modrego et al., 2006</td>
<td>Riv</td>
<td>24</td>
<td>16</td>
<td>Frontal cortex</td>
<td>Increased NAA/tCr vs baseline</td>
<td>+5.7%</td>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td>Occipital cortex</td>
<td>Increased mI/tCr vs baseline</td>
<td></td>
</tr>
<tr>
<td>Krishnan et al., 2003</td>
<td>Don</td>
<td>28</td>
<td>12&amp;18</td>
<td>Periventricular white matter</td>
<td>Increased NAA vs control</td>
<td>N.C.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Subcortical gray matter</td>
<td></td>
<td></td>
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<td></td>
<td></td>
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</tbody>
</table>

ChEI - cholinesterase inhibitor, N - number of participants, Int. - treatment interval (weeks), Δ - change in, Don - donepezil, PC - posterior cingulate, Gal - galantamine, hipp - hippocampus, Riv - rivastigmine, N.C. - no change over time

The posterior cingulate is of particular interest in AD for several reasons. First, cholinergic axons, implicated in the pathology of AD, project directly from the basal forebrain to the posterior cingulate cortex (24,47-49). Second, the posterior cingulate is a site of known pathology including neuronal loss (50,51) and β-amyloid accumulation (52). Furthermore, numerous imaging modalities have reported changes in the posterior cingulate.
in AD patients, including MRS (36,46,53-55), PET (52), and fMRI blood flow (56). Finally, the location of the posterior cingulate within the brain makes it amenable to the acquisition of high quality $^1$H MRS data (57). As high magnetic field strength ($\geq 3$T) magnetic resonance imaging (MRI) systems become more accessible $^1$H MRS spectra can be acquired with greater signal-to-noise ratio and improved spectral resolution (58-60), increasing the sensitivity of this technique.

The purpose of this study was to determine whether four months of rivastigmine treatment would alter metabolite levels measured by $^1$H MRS in the bilateral posterior cingulate cortex of subjects with AD, and to correlate metabolic changes with standard cognitive assessment scores. Based on previous studies, we hypothesized that four months of rivastigmine treatment would result in increased levels of NAA and NAA/tCr measured from the bilateral posterior cingulate cortex of patients with AD.

### 3.2 Method

#### 3.2.1 Patient population

This study was approved by the Western University Health Sciences Research Ethics Board. Sixteen subjects were recruited from the Aging Brain and Memory Clinic in London, Ontario, Canada. All subjects were newly diagnosed with probable AD using the National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer’s Disease and Related Disorders Association (NINCDS/ADRDA) criteria. Nine subjects had at least one vascular risk factor including six with hypertension, eight with hyperlipidemia, and one with diabetes; all were stable on medication. All participants gave written informed consent. At
follow-up, one subject received a new diagnosis of Primary Progressive Aphasia and was removed from the study. The data from another subject was not included in the analysis because their $^1$H MRS spectrum at follow-up had insufficient signal-to-noise ratio (SNR < 5). Of the fourteen subjects (70.4 ± 9.3 years of age, 12 right handed, 7/7 female/male) included in the analysis, thirteen were diagnosed with mild AD and one with moderate AD at baseline. Cognitive testing included the Mini-Mental State Examination (MMSE) (1), the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) (2), and the neuropsychiatric inventory (NPI) (61), and were administered to the participants immediately before (baseline) and four months after beginning rivastigmine treatment via the transdermal patch. All subject and caregiver dyads were instructed on the location of patch application based on the standard product monograph and reported toleration and daily compliance. After the baseline cognitive tests and MRI scan session were completed, subjects were started on the rivastigmine patch (4.6 mg/24 hours for the first month, 9.5 mg/24 hours thereafter). This two-step dosing approach mirrored the same two-step approach previously used to study the effects of donepezil (32) and galantamine (38). $^1$H MRS was used to measure the concentrations of metabolites within the bilateral posterior cingulate cortex of all subjects at baseline and again after four months of treatment. Table 3.2 summarizes the study participant demographics. A non-treatment AD control group was not included in this study because as recommended at the Third Canadian Consensus Conference on Diagnosis and Treatment of Dementia (3rd CCCDTD, 2006), “provision of the best standard of care for the patient must always remain the priority” (62), and therefore withholding the standard treatment was deemed unethical. Instead, we used previously reported metabolite level longitudinal
measurements in AD patients that were drug naïve or stable on medication to compare to our cohort.

Table 3.2: Study participant demographic data and cognitive test scores

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>4 months</th>
<th>p-value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>14</td>
<td>14</td>
<td>-</td>
</tr>
<tr>
<td>Gender</td>
<td>7F/7M</td>
<td>7F/7M</td>
<td>-</td>
</tr>
<tr>
<td>Age (years)</td>
<td>70.4 ± 9.3</td>
<td>70.7 ± 9.3</td>
<td>-</td>
</tr>
<tr>
<td>Education (years)</td>
<td>13.9 ± 2.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>AD severity</td>
<td>13 mild/1 moderate</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Family history of AD</td>
<td>10 yes/4 no</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Vascular risk factors</td>
<td>9 yes/ 5 no</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Concomitant meds</td>
<td>10 yes/4 no</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MMSE</td>
<td>24.9 ± 3.6</td>
<td>25.1 ± 3.3</td>
<td>0.74</td>
</tr>
<tr>
<td>ADAS-cog</td>
<td>20.4 ± 12.7</td>
<td>19.1 ± 13.0</td>
<td>0.49</td>
</tr>
<tr>
<td>NPI</td>
<td>4.8 ± 6.6</td>
<td>6.1 ± 7.3</td>
<td>0.30</td>
</tr>
</tbody>
</table>

Age, Education, MMSE, ADAS-cog, and NPI values listed as mean ± standard deviation
# - p-values for two-tailed repeated measures t-tests
N - number of participants, F - female, M - male
3.2.2 Magnetic resonance imaging and spectroscopy

All imaging and $^1$H MR spectroscopic data were acquired using a 3T Siemens Magnetom Tim Trio whole-body MRI with a 12-channel head coil. Sagittal T$_1$-weighted 3D magnetization prepared rapid gradient echo (MPRAGE) ($1 \times 1 \times 1.2$ mm$^3$, TR/TE = 2300/2.86 ms, TI = 900 ms) images (Figure 3.1a) and axial T$_2$-weighted 2D turbo spin-echo (TSE) ($0.9 \times 0.9 \times 3$ mm$^3$, TR/TE = 3000/99 ms) images (Figure 3.1b) were acquired for each scan and were subsequently used for $^1$H MRS voxel (measurement volume) placement.

Figure 3.1: Sagittal T$_1$-weighted 3D MPRAGE ($1 \times 1 \times 1.2$ mm$^3$, TR/TE/TI = 2300/2.86/900 ms) image (a) and axial T$_2$-weighted 2D TSE ($0.9 \times 0.9 \times 3$ mm$^3$, TR/TE = 3000/99 ms) image (b) of a subject with Alzheimer disease acquired on a Siemens 3T MRI system. The $^1$H MRS voxel (white box) is positioned over the bilateral posterior cingulate cortex.
Single-voxel $^1$H MRS data were acquired from a 2x2x2 cm$^3$ volume of interest in the bilateral posterior cingulate cortex (Figure 3.1) at baseline and after four months of treatment. A point resolved spectroscopy (PRESS) (TR/TE = 2000/135 ms) sequence was used to localize $^1$H MRS data acquisition, which included 192 water suppressed averages and 8 unsuppressed averages for each scan. Data were lineshape corrected using the QUECC method (63), to correct for eddy current and magnetic field inhomogeneity distortions. Any remaining unsuppressed water signal present in the suppressed spectrum was removed by subtracting resonances between 4.1 and 5.1 parts per million (ppm) (water ~ 4.7 ppm) as determined by an automated Hankel singular value decomposition (HSVD) algorithm (64).

### 3.2.3 Data analysis and statistics

The resulting water-removed metabolite spectra were fitted using a Levenberg-Marquardt minimization routine in the time domain (fitMAN software (65)), which incorporated a template of prior knowledge of metabolite lineshapes as previously described (64,65). $N$-acetylaspartate (NAA), glutamate (Glu), glutamine (Gln), total creatine ($t$Cr = Cr + PCr), choline (Cho = GPC + PC), and myo-inositol (mI) were included in the fitting template because previous studies have implicated these metabolites in AD pathophysiology, and because these metabolites could be reliably measured. The concentrations of NAA, Glx (Glu+Gln), $t$Cr, Cho, and mI and the ratios of NAA/$t$Cr, Glx/$t$Cr, Cho/$t$Cr, and mI/$t$Cr were calculated and included in the statistical analysis. Ratios relative to $t$Cr were included as they are commonly reported in the literature and may be sensitive indicators when the two metabolites in the ratio are changing in opposing directions. Also, ratios are not vulnerable to
measurement errors caused by imperfect partial volume and relaxation corrections (34).

Absolute metabolite levels were calculated by scaling the metabolite spectral areas to the unsuppressed water signal within the voxel and correcting signal amplitudes for T₁ and T₂ relaxation as previously described (64). Water content was estimated as 81% in gray matter and 71% in white matter (66). In the current study the proportion of gray matter, white matter, and cerebral spinal fluid within the ¹H MRS measurement volume was determined by segmenting the T₁-weighted MPRAGE images using FMRIB’s Automated Segmentation Tool (FAST) (67) available in FMRIB’s Software Library (FSL) (68-70).

Paired two-tailed repeated measures t-tests were used to compare baseline and four month concentrations of NAA, Glx, tCr, Cho, and mI as well as ratios of NAA/tCr, Glx/tCr, Cho/tCr, and mI/tCr, and the MMSE, ADAS-cog, and NPI scores. For all concentrations, ratios, and cognitive scores, significance was established at p < 0.05. Furthermore, correlation analysis was used to compare baseline concentrations and ratios to baseline cognitive scores and changes in cognitive scores, and to compare the change in concentrations and ratios to the change in cognitive scores. All statistical tests were performed in Prism (GraphPad Software, Inc., Version 6.0c, La Jolla, CA).

### 3.3 Results

The study participant demographics and clinical data are summarized in Table 3.2. There were no significant differences in average MMSE, ADAS-cog, or NPI scores between baseline and 4-month follow-up testing sessions. Water and metabolite (water-suppressed) spectra were successfully acquired at baseline and at 4-month follow-up in fourteen subjects.
Figure 3.1 shows typical sagittal and axial images used to position the $^1$H MRS voxel over the bilateral posterior cingulate cortex. The metabolite spectrum acquired from one subject at baseline is shown in Figure 3.2, along with the fitted result and the residual difference between the fit and the data. Figure 3.2 also shows the individual spectral components of NAA, Glu, Gln, tCr, Cho, and mI that contributed to the fitted spectrum. The average proportions of gray matter, white matter, and cerebral spinal fluid within the $^1$H MRS measurement volumes were 48.2%, 26.2%, and 25.7% respectively at baseline and 49.8%, 25.6%, and 24.6% respectively at four months. There were no statistical differences in these values between baseline and 4-month follow-up.
Figure 3.2: A representative baseline spectrum from one subject with Alzheimer disease, with the fitted result (black line) superimposed onto the acquired data (in gray). The residual difference between the data and the fit is shown above the spectrum, and the individual metabolite spectra are shown beneath.

There were no significant differences in average metabolite concentrations between baseline and 4-month measurements (Figure 3.3).
Figure 3.3: Average metabolite concentrations at baseline (BL) and after four months (4m) of rivastigmine treatment in 14 patients with Alzheimer disease. Error bars represent the standard error of the mean.

Average metabolite ratios normalized to tCr are shown in Figure 3.4, for baseline and 4-month measurements. The NAA/tCr values were normally distributed, as determined by a D’Agostino-Pearson omnibus normality test, permitting the use of a repeated measures t-test to compare metabolite values between time points. The ratio of NAA/tCr decreased by 12.7% ($t = -3.274$, df = 13, $p < 0.01$) between baseline and 4-month $^1$H MRS scans, while no other ratio changed significantly.
Figure 3.4: Average metabolite ratios at baseline (BL) and after four months (4m) of rivastigmine treatment in 14 patients with Alzheimer disease. Error bars represent the standard error of the mean.

There were no significant correlations between any baseline metabolite concentration or ratio and any baseline cognitive score, or between any baseline metabolite concentration or ratio and any change in cognitive score. However, over the four months of treatment, significant correlations were observed between the change ($\Delta$) in NAA/tCr and the $\Delta$MMSE ($F = 7.38$, df = 12, $p < 0.05$, Figure 3.5a), and between $\Delta$Glx/tCr and the $\Delta$MMSE ($F = 7.47$, df = 12, $p < 0.05$, Figure 3.5b).
Figure 3.5: The associations between the change in the ratio of NAA/tCr and the change in MMSE (a), and between the change in the ratio of Glx/tCr and the change in MMSE (b). The black lines represent the linear fit to the data.

No other concentration change or ratio change correlated with changes in cognitive score.

There was also a correlation between $\Delta$NAA/tCr and $\Delta$Glx/tCr ($F = 6.00$, $df = 12$, $p < 0.05$, Figure 3.6), even though Glx/tCr did not change significantly over four months of treatment.

Figure 3.6: The association between the change in the ratio of NAA/tCr and the change in the ratio of Glx/tCr.
3.4 Discussion

The purpose of this study was to measure metabolite levels using $^1$H MRS within the bilateral posterior cingulate cortex in subjects recently diagnosed with AD in response to four months of rivastigmine treatment, and to determine whether metabolite levels were correlated with cognitive performance. This study is the first to measure the effects of rivastigmine treatment on metabolite levels in the posterior cingulate cortex. Although a change in cognitive score was not detected across the group, the average ratio of NAA/tCr was found to decrease in subjects with AD following four months of rivastigmine treatment. More importantly, the change in NAA/tCr over the four months of treatment was positively correlated with the change in MMSE score, indicating that there was less of a decline in NAA/tCr in subjects with cognitive improvement following treatment compared to subjects with cognitive decline.

Deficits in the cholinergic neurotransmitter acetylcholine (ACh) have been consistently reported in AD following the degeneration of cholinergic neurons originating in the basal forebrain that project to the cortex and hippocampus (23). The use of ChEI treatment generally improves cognitive performance over the first 6-12 months of treatment (25-28). For example Wattmo and colleagues measured the cognitive response to ChEI treatment in 843 subjects with AD every six months for three years (71). They reported cognitive improvement, as measured by the MMSE and ADAS-cog, between zero and six months followed by cognitive decline thereafter, indicating that the greatest cognitive benefits from ChEIs occur within six months of starting treatment. Therefore it is important to rapidly assess therapeutic response so that individual treatment can be optimized.
There are currently seven studies in the literature that have reported metabolite level changes in subjects with AD in response to ChEI treatment (summarized in Table 3.1). Four of these were longitudinal donepezil treatment studies that found: NAA/tCr increased in the dorsolateral prefrontal cortex (33) and bilateral posterior cingulate (36); NAA/tCr decreased in the left prefrontal region (36) and right hippocampus (32); a correlation between the change in NAA and the change in ADAS-cog in the parietal lobe (34); and increased ml/tCr in the left occipital lobe and right prefrontal region (36) and decreased ml/tCr in the right hippocampus (32). A fifth donepezil study found increased NAA in the periventricular white matter and subcortical gray matter in subjects with AD compared to control (35). One longitudinal rivastigmine study reported increased NAA/tCr in the frontal cortex and increased ml/tCr in the occipital cortex (37). The only longitudinal galantamine study to date found increased Glu/tCr in the right hippocampus that correlated with MMSE scores (38) and no change in NAA/tCr ratio. In summary, NAA/tCr was increased in seven regions and decreased in three regions, while ml/tCr was increased in three regions and decreased in one region. The one study that reported changes in Glu found an increase in Glu/tCr that correlated with MMSE, indicating a beneficial effect in the right hippocampus following galantamine treatment. These studies indicate region-specific effects of ChEI treatment. However it is also possible that the discrepancies in the reported ratio changes may be due to any combination of differences in cohorts, field strengths, duration of treatment, and spectral quantification methodology. Interestingly, a previous study performed by our group to examine the effect of four months of donepezil treatment also found that NAA/tCr decreased by 16% in the right hippocampus in subjects with AD after four months of treatment (32).
The large decrease in NAA/tCr found in the current study cannot be explained by atrophy since the effect of tissue partial volume within the voxel was eliminated by taking the ratio of metabolite levels to tCr. Also, it should be noted that test-retest reliability measurements of NAA/Cr made in a group of young healthy controls using the same methodology as that described in the current manuscript demonstrate no changes over a period of six months (p > 0.7, data not shown). An increase in the absolute level of tCr could contribute to the large observed decrease in NAA/tCr. However, in a previous study by our group the 16% decrease in NAA/tCr over 4 months in AD subjects receiving donepezil treatment (32) was attributed to a 13% decrease in NAA while a significant increase in tCr was not observed. Similarly, in the current study there was neither a significant decrease in absolute NAA or a significant increase in absolute tCr (Figure 3.4).

The one other 1H MRS rivastigmine study to date found increased NAA/tCr in the frontal cortex and increased mI/tCr in the occipital cortex after four months of treatment (37), while the current study found a decrease in NAA/tCr in the bilateral posterior cingulate cortex, indicating a differential effect of brain region. Furthermore, a previous study that reported changes in bilateral posterior cingulate found an increase in NAA/tCr following donepezil treatment, indicating a differential effect between donepezil and rivastigmine treatment.

Previous 1H MRS studies of subjects with AD that are not receiving treatment or that are stable on treatment have reported decreased NAA or NAA/tCr compared to control subjects (40-46,54) including in the posterior cingulate cortex (46,54). These studies are summarized in Table 3.3, for comparison to the current treatment study.
### Table 3.3: Summary of previously reported longitudinal NAA findings in AD

<table>
<thead>
<tr>
<th>Study</th>
<th>% on ChEI</th>
<th>N</th>
<th>Interval</th>
<th>Location</th>
<th>NAA findings</th>
<th>Interpolated NAA change over 4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Schott et al., 2010</td>
<td>71</td>
<td>42</td>
<td>24 months</td>
<td>Midline PC</td>
<td>NAA/tCr decreased by 2.2% per year</td>
<td>-0.73%</td>
</tr>
<tr>
<td>Kantarcı et al., 2007</td>
<td>75</td>
<td>60</td>
<td>13 months</td>
<td>Bilateral PC gyri</td>
<td>NAA/tCr decreased by 1.8% over 13 months</td>
<td>-0.55%</td>
</tr>
<tr>
<td>Dixon et al., 2002</td>
<td>NR</td>
<td>9</td>
<td>12 months</td>
<td>Left and right hippocampus</td>
<td>No longitudinal change</td>
<td>N.C.</td>
</tr>
<tr>
<td>Jessen et al., 2001</td>
<td>NR</td>
<td>13</td>
<td>23 months</td>
<td>Medial temporal lobe</td>
<td>No longitudinal change NAA/tCr correlated with MMSE</td>
<td>N.C.</td>
</tr>
<tr>
<td>Adalsteinsson et al., 2000</td>
<td>12</td>
<td>12 months</td>
<td>Averaged grey matter</td>
<td>NAA decreased by 12.4% over 1 year</td>
<td>-4.1%</td>
<td></td>
</tr>
</tbody>
</table>

average = -1.8%

* - percent of subjects on cholinesterase inhibitor treatment at baseline
N - number of participants, PC - posterior cingulate, NR - not reported, N.C. - no change over time

The three previous studies that reported longitudinal changes in NAA/tCr all reported decreases over time (42,72,73), with an interpolated average 1.8% decline in NAA/tCr over four months. The 12.7% decrease in NAA/tCr over four months observed in the current study is greater than expected based on these previous studies. However, it is important to note that we measured the short-term effects of treatment, as all subjects were treatment naïve at the baseline measurement and were then treated for 4 months. Conversely, most subjects in the studies summarized in Table 3.3 were either stable on treatment or untreated and the time between measurements was typically 12-24 months. Therefore, short-term changes due to
treatment would not be detected.

It is worth noting that the decrease in NAA/tCr found in the current study could not be attributed to a significant decline in absolute NAA or a significant increase in absolute tCr and therefore provides an example of the potential increase in sensitivity of metabolic ratios. Both NAA/tCr and Glx/tCr were positively correlated with the MMSE score. This result indicates that maintained or improved cognitive performance is associated with preserved neuronal function. It is not possible to determine whether this effect is due to rivastigmine treatment or disease progression but regardless suggests that the NAA/tCr ratio is an objective measure of cognitive function following symptomatic drug treatment.

The limitations of this study include the small sample size (fourteen subjects) and the acquisition of $^1$H MRS data from only one brain region. Future studies would benefit from investigating multiple regions that are involved in progressive AD, as well as control regions. However the posterior cingulate cortex region used in the current study should be considered for future multi-site studies as the region is implicated in the progression of Alzheimer’s disease and allows high quality reproducible metabolite level measurements. Follow-up with longer treatment duration may also provide additional insight into whether rivastigmine treatment maintains NAA/tCr and Glx/tCr levels and cognition.
3.5 Acknowledgments

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3.6 Statement of Interest

All authors report no conflicts of interest directly related to the content of this manuscript. Unrelated to this manuscript: Michael Borrie has received speaker fees from Merck and consultation fees from Eli Lilly and Merck, Jennie Wells will receive speaker fees from Merck, and Robert Bartha is the Chief Scientific Officer and an equity holder in Bioscape Imaging Solutions Inc.
3.7 References


Chapter 4

Semi-LASER $^1$H MR spectroscopy at 7 Tesla in human brain: metabolite quantification incorporating subject-specific macromolecule removal

4.1 Introduction

Proton (\(^1\)H) magnetic resonance spectroscopy (MRS) has been used extensively to detect metabolite levels \textit{in vivo} in various neuropathological conditions in the brain (1-6). Most clinical studies use long echo times (TE > 100 ms) to detect changes in \(N\)-acetylaspartate (NAA), total creatine (tCr = Cr + PCr), and choline (Cho = GPC + PC). Long-TE spectra (e.g. TE = 135 ms, 270 ms) are relatively simple to analyze as they contain easily resolved peaks from NAA, tCr, and Cho and few overlapping signals from macromolecules (7). In contrast, acquisition with short-TE (TE < 50 ms) reduces signal loss from J-coupling phase modulation producing visible multiplets from metabolites including glutamate (Glu), glutamine (Gln), and myo-inositol (ml). However, quantification of all metabolites becomes more difficult due to overlapping macromolecule signals (7) that must be resolved.

The \textit{in vivo} spectrum can be fitted using linear combinations of known metabolite lineshapes (8). This approach has been successfully demonstrated by several groups at field strengths ranging from 1.5T to 9.4T (8-16). However, the method is only accurate when all contributions to the \textit{in vivo} spectrum are incorporated during fitting (12), including macromolecule resonances that extend beneath the 2-4 ppm metabolite region and overlap with metabolites (e.g. NAA, Glu, Gln, and tCr). One approach to account for the macromolecule contribution is to include prior information about the macromolecule lineshapes in the fitting (17). However, this becomes difficult as macromolecule contributions to the spectrum have been shown to change in disease conditions including ischemic stroke (18) and cancer (19,20) as mobile lipids become MR visible, potentially
leading to inaccurate metabolite measurements (21). Furthermore, Opstad et al. (19) showed that the macromolecule signals differ substantially between tumor types.

Alternatively, a subject-specific macromolecule spectrum can be acquired in addition to the metabolite spectrum, and subsequently subtracted from the metabolite spectrum to remove the macromolecule contributions (21). Although this approach increases acquisition time and reduces signal to noise ratio due to the subtraction of one spectrum from the other (21), it provides the most direct and accurate method to identify subject specific macromolecule contributions. The longitudinal relaxation times ($T_1$) of macromolecules are generally shorter ($T_1 \sim 430$ ms at 7T (22)) than metabolites ($T_1 \sim 1600$ ms at 7T (22)), allowing for the direct measurement of the macromolecule spectrum by inversion-nulling the metabolite signals (23,24). However, the inversion-nulling scheme must be optimized for a given field strength because the $T_1$ time constants of the metabolites are field strength dependent.

Signals arising from outside the volume of interest must also be minimized. For example, outer volume saturation (OVS) can be used to reduce signals from outside of the measurement volume that may contaminate the spectrum. However, spoiling of unwanted signals from outside of the measurement volume is often not sufficient to eliminate lipid contamination in single-volume stimulated-echo acquisition mode (STEAM) spectroscopy (24). Furthermore, OVS can decrease the SNR of metabolites within the measurement volume due to magnetization transfer effects (25). Alternatively, the use of localization by adiabatic selective refocusing (LASER) decreases the need for OVS due to the excellent slice-selection profiles obtained when using pairs of adiabatic full passage (AFP) pulses (26);
a significant advantage for high field applications.

The use of ultra-high magnetic field strength (e.g. ≥7T) produces $^1$H MRS spectra with greater signal-to-noise ratio (SNR) and improved spectral resolution (15,27-32), generally improving overall metabolite quantification. However, power deposition also increases due to the increase in resonance frequency, and $B_0$ and $B_1^+$ (RF transmit field) magnetic field homogeneity is difficult to achieve. These factors must all be considered when designing the optimal spectroscopy protocol. Spectra can be acquired with a semi-LASER sequence (33-35), rather than the LASER pulse sequence, to significantly reduce power deposition (33-35). Furthermore, $B_0$ homogeneity can be optimized with an automated shimming technique such as RASTAMAP (36), and $B_1^+$ can be optimized using a parallel transmission shimming protocol (37) that maximizes excitation efficiency based on $B_1^+$ amplitude and uniformity while minimizing the specific absorption rate (SAR).

The purpose of this study was to develop an in vivo $^1$H short-TE MRS protocol at 7T to reliably quantify absolute metabolite concentrations in the brain. This goal was achieved incorporating parallel transmit excitation, subject-specific macromolecule removal, and tissue partial volume measurements.

4.2 Methods

4.2.1 General MRS protocol

An Agilent 7 Tesla head-only MRI scanner (Agilent Inc, Walnut Creek, CA) with a Siemens AC84 7T gradient coil (Siemens, Erlangen, Germany) was used to acquire all imaging and spectroscopic data. At the time of data collection the system had sixteen
controllers driving sixteen radiofrequency (RF) transmit/receive channels. Prior to the acquisition of imaging data, linear and higher-order shims were used to optimize the static magnetic field ($B_0$) over the entire head (global) using an automated shimming sequence (RASTAMAP) (36). After the imaging data were acquired, RASTAMAP was used again to maximize $B_0$ homogeneity within the MRS voxel. Following the initial global $B_0$ shim and before images were acquired, $B_1^+$ maps were produced for each of the 16 transmitters. These maps were then used to optimize the overall $B_1^+$ power and uniformity, while minimizing SAR, with a slice-by-slice parallel transmit shimming protocol (37) that modulated the driving amplitudes and phases of the separate transmit elements. This process occurred over the entire head prior to imaging, and again over the voxel prior to MRS data collection (37).

MRS data were acquired with a semi-LASER (33-35) sequence that is based on the original LASER sequence (26) but with a single slice-selective 90° excitation pulse used in place of the original non-selective 90° excitation pulse and the first pair of 180° adiabatic full passage (AFP) refocusing pulses. A 2 ms slice-selective sinc pulse was used for excitation and 3.4 ms hyperbolic secant AFP (HS2 R10, 3.2 kHz bandwidth) pulses were used for selective-refocusing in the remaining two orthogonal planes. The pulse power levels were manually calibrated to ensure pulses were optimized. The optimal adiabatic pulse power was determined by acquiring a water unsuppressed spectrum repeatedly while increasing the RF power until the amplitude of the water spectrum reached and maintained a maximum value. The power corresponding to the first instance of the maximum signal intensity was identified and this value plus 1 dB was used in the data acquisition to ensure the adiabatic condition was satisfied. The semi-LASER sequence was used as it offers a shorter echo-time (TE) and
lower RF power deposition than the original LASER sequence because of the removal of one pair of refocusing pulses. The water signal was suppressed with variable pulse power and optimized relaxation delays (VAPOR) water suppression (17) with one additional pulse after the seventh VAPOR pulse, adjusted to yield optimal in vivo water suppression. The macromolecule spectra were acquired by nulling the metabolite signals; this was achieved using a double-inversion nulling method (38) that utilized two AFP (HS2 R10, 5 ms, 2.2 kHz bandwidth) pulses. Double-inversion was used because one inversion pulse can only fully suppress signals that relax with one specific T₁ value. At 7T the T₁s of metabolites in the brain range from 1.1-2.1s (22). Therefore, the use of the double inversion-nulling improves the nulling efficiency of metabolites with a range of T₁ time constants.

The 16-channel transmit/receive phased-array radiofrequency (RF) coil used to acquire the MRS data produced sixteen spectra per acquisition. These spectra were combined using an in-house script written in Matlab (The Mathworks Inc., Natick, MA) as described in Brown et. al. (39), to produce a single spectrum per acquisition. The method used a coil sensitivity weighting factor (wᵣ) computed from the noise in each channel, a coil proximity weighting factor (aᵣ) determined from the maximum amplitude of the signal from each channel, and a phase compensation factor (φᵣ) to align the phases in all sixteen channels.

Lineshape distortions that arise from magnetic field inhomogeneities and induced eddy currents and lead to errors in spectral peak area estimations were corrected with QUAntification improvement by converting LIneshapes to the lorentzian TYpe (QUALITY) deconvolution (40) performed on the initial 200 complex time domain data points (50 ms) followed by eddy current correction (ECC) (41) on the remaining 1800 data points (450 ms).
This combined QUALITY/ECC post-processing (QUECC,(27)) uses the unsuppressed water time domain data as the reference signal to correct the metabolite data. The QUECC junction was set to 50 ms. All residual water in the spectrum was also removed prior to fitting the metabolite signals. Water was removed by subtracting all resonances found between 4.2 and 6.2 ppm (water at 4.7 ppm) following an automated Hankel singular value decomposition (HSVD) fit (512 points) of the data (9,10,42,43).

The processed metabolite spectra were fitted in the time domain using the fitMAN software (9,10). Specifically, a Levenberg-Marquardt minimization routine (44) was used that incorporated prior knowledge from sixteen metabolite lineshapes including: N-acetylaspartate (NAA), N-acetylaspartylglutamate (NAAG), γ-aminobutyric acid (GABA), aspartate (Asp), creatine (Cr), glucose (Glc), glutamate (Glu), glutamine (Gln), glutathione (Glth), glycerophosphorylcholine (GPC), myo-inositol (mI), phosphocreatine (PCr), phosphorylcholine (PC), phosphorylethanolamine (PEth), scyllo-inositol (Scy), and taurine (Tau). All metabolites were purchased from Sigma-Aldrich Canada Co. (Oakville, Ontario). Alanine and lactate were excluded from the current study as they could not be fit reliably. Prior knowledge regarding the lineshape of each metabolite was obtained as previously described (10), using the identical semi-LASER pulse sequence used to acquire all in vivo data. More specifically, high-resolution in vitro spectra (11,14) were acquired from aqueous solutions of each metabolite (100 mM each, except NAAG which had a concentration of 5.1 mM due to the cost of NAAG, all pH adjusted to 7.04). Each solution contained sodium 3-trimethylsilyl-propionic acid (TSP) as a chemical shift and Lorentzian damping reference (10). The chemical shift and Lorentzian damping of each resonance within each metabolite
was calculated relative to the TSP resonance to eliminate $B_0$ and magnetic field homogeneity dependencies in the fitting template. The chemical shifts and Lorentzian dampings of all metabolites were each fixed to a common parameter and the resonance amplitudes for a given metabolite were fixed to a relative amplitude parameter, so that the relative amplitude of each metabolite was free to be adjusted during the fitting process (10).

4.2.2 Optimal inversion times for metabolite suppression

Accurate measurement of the macromolecule baseline in each subject required suppression of metabolite signals. This suppression was achieved with a double inversion nulling method (38) that used two AFP inversion pulses (5 ms, HS2 R10, 2.2 kHz bandwidth) at inversion times $T_{I1}$ (time between the first inversion pulse and the 90° excitation pulse in the semi-LASER sequence), and $T_{I2}$ (time between the second inversion pulse and the 90° excitation pulse in the semi-LASER sequence). To determine the optimal inversion times ($T_{I1}$ and $T_{I2}$) for metabolite suppression, the longitudinal relaxation times ($T_1$) of NAA, Glu, tCr, and Cho were measured from a 3x3x3 cm$^3$ voxel in the parietal-occipital region (Figure 4.1) of seven healthy volunteers (27.1 ± 2.4 years) using a research protocol approved by with the University of Western Ontario Health Sciences Research Ethics Board.

The semi-LASER sequence was preceded by a single AFP inversion pulse (5 ms, HS2 R10, 2.2 kHz bandwidth), and a water suppressed spectrum was acquired at as many of the following inversion times (TI) as time allowed in each subject: 140, 240, 340, 640, 1140, 2140, 2640, 3140, 3840, 4040, 4440 ms (TE/TR = 38/5000 ms, receiver bandwidth = 4 kHz,
voxel volume = 27 cm$^3$, 32 averages). An unsuppressed water spectrum (TE/TR = 38/5000 ms, receiver bandwidth = 4 kHz, voxel volume = 27 cm$^3$, 8 averages) was also acquired in each subject and used as a reference for QUECC correction (50 ms QUECC junction).

Figure 4.1: Axial T$_2$-weighted 2D FLASH image (TR/TE = 1000/6.5 ms, 1x1x2 mm$^3$) with the 3x3x3 cm$^3$ voxel (white square) used to determine the optimal inversion times for metabolite suppression and the 2x2x2 cm$^3$ voxel (dashed white square) used to determine the in vivo metabolite concentrations, both in the parietal-occipital region.

To reduce the influence of the broad (short T$_2$) macromolecule signals on the measurement of metabolite T$_1$, a set number of data points were omitted from the beginning of each time-domain data set when fitting in the time domain. It was empirically determined that 14 points (0.0035s) provided the best compromise between maintaining spectral signal to noise ratio
and removing macromolecules signals that contaminated the measured metabolite areas. For the measurement of $T_1$, the amplitude of the tCr$_{CH3}$ and Cho$_{CH3}$ peaks were fit independently from the other peaks in the metabolite spectrum. Using this approach, inversion recovery curves were produced for NAA, Glu, tCr (CH$_3$ at 3.03 ppm), and Cho (CH$_3$ at 3.19 ppm) by plotting their fit contributions, $M_d(t)$, at each inversion time (TI), and then the data were fitted to an exponential regrowth equation (Equation 4.1) using Prism (GraphPad Software, Inc., Version 6.0c, La Jolla, CA) (Figure 4.2);

$$M_z(TI) = M_0 \left( 1 - e^{-TI/T_1} - ae^{-TI/T_1} + ae^{-TR/T_1} \right)$$  \hspace{1cm} (4.1)

where $M_0$ is the steady-state metabolite signal and $a$ is the inversion fraction following imperfect inversion, such that $M_d(0) = -aM_0(1 - \exp(-TR/T_1))$. The fit directly yielded the longitudinal relaxation time constants ($T_1$) listed in Table 4.1.
Figure 4.2: The inversion recovery curves for NAA, Glu, tCrCH₃, and ChoCH₃. Each curve was fitted to the single inversion recovery equation (Equation 4.1 - black lines) to solve for the T₁ time constant.
Table 4.1: $T_1$ and $T_2$ time constants used to calculate metabolite mM concentrations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>$T_1$ (s)</th>
<th>Reference</th>
<th>$T_2$ (ms)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tissue Water</td>
<td>1.70</td>
<td>(45)</td>
<td>52.5</td>
<td>(46)</td>
</tr>
<tr>
<td>CSF Water</td>
<td>4.43</td>
<td>(47)</td>
<td>141</td>
<td>(46)</td>
</tr>
<tr>
<td>NAA</td>
<td>1.71 ± 0.15</td>
<td>Current study</td>
<td>341</td>
<td>(48)</td>
</tr>
<tr>
<td>NAAG</td>
<td>1.21</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>Glu/Gln</td>
<td>1.68 ± 0.19</td>
<td>Current study</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>Cr/PCr (3.03 ppm)</td>
<td>1.63 ± 0.10</td>
<td>Current study</td>
<td>221</td>
<td>(48)</td>
</tr>
<tr>
<td>Cr/PCr (3.92 ppm)</td>
<td>1.13</td>
<td>(22)</td>
<td>221</td>
<td>(48)</td>
</tr>
<tr>
<td>PC/GPC (3.19 ppm)</td>
<td>1.41 ± 0.09</td>
<td>Current study</td>
<td>260</td>
<td>(48)</td>
</tr>
<tr>
<td>Glut</td>
<td>1.14</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>mI</td>
<td>1.28</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>PEth</td>
<td>1.31</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>Scy</td>
<td>1.31</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>Tau</td>
<td>2.15</td>
<td>(22)</td>
<td>274</td>
<td>Avg</td>
</tr>
<tr>
<td>Other</td>
<td>1.61</td>
<td>Avg</td>
<td>274</td>
<td>Avg</td>
</tr>
</tbody>
</table>

Avg - Relaxation time was set as the average of NAA, Glu, tCr (3.03 ppm), and Cho. $T_1$s from the current study listed with ± standard error of the fit.

The signal equation following double inversion (Equation 4.2) was then used to model $M_z$ as a function of $T_{I1}$ and $T_{I2}$ for each metabolite (NAA, Glu, tCr, and Cho).

$$M_z(TI_1, TI_2) = M_0 \left(1 - 2e^{-TI_2/T_1} + 2e^{-TI_1/T_1} - e^{-TR/T_1}\right)$$  \hspace{1cm} (4.2)

To suppress a metabolite signal $M_z(TI_1, TI_2)$ was set to zero in Equation 4.2, and then
rearranged to give Equation 4.3.

\[
\text{T}_{I_2} = -T_1 \ln \left( \frac{1}{2} + e^{-T_1/T_{T1}} - \frac{1}{2} e^{-T_R/T_{T1}} \right)
\]  

(4.3)

The \(T_1\) values for NAA, Glu, tCr, and Cho were each substituted into Equation 4.3, producing four equations of \(T_{I_2}\) versus \(T_{I_1}\) that are plotted in Figure 4.3. Each one of these curves defines the \(T_{I_1}\) and \(T_{I_2}\) values that will null that respective metabolite.

Figure 4.3: \(T_{I_1}\) versus \(T_{I_2}\) curves for NAA, Glu, tCr\(_{CH3}\), and Cho\(_{CH3}\). Each of these curves defines \(T_{I_1}\) and \(T_{I_2}\) values that will null that respective metabolite. A single set of \(T_{I_1}\) and \(T_{I_2}\) values at the point closest to the intersection of all four curves (see zoomed overlay), results in optimal simultaneous suppression of all four metabolites.
4.2.3 Average metabolite concentrations in healthy brain

Five healthy volunteers (28.0 ± 2.7 years) were scanned using a research protocol approved by the University of Western Ontario Health Sciences Research Ethics Board. To begin each scan session: 3 localizer images were acquired, an automated global B\textsubscript{0} shim (36) was performed, and B\textsubscript{1}\textsuperscript{+} (transmit magnetic field) maps were acquired for subsequent B\textsubscript{1}\textsuperscript{+} shimming (37). T\textsubscript{2}-weighted 2D FLASH (Fast Low Angle SHot) images (Figure 4.1) were acquired (TE = 5.5 ms, TR = 1000 ms, flip angle = 30°, 1x1 mm\textsuperscript{2} in-plane resolution, slice thickness = 2 mm, scan time = 3 minutes) for planning the MRS voxel: a 2x2x2 cm\textsuperscript{3} volume positioned in the bilateral parietal-occipital cortex (Figure 4.1) in each participant. In preparation for MRS data acquisition the B\textsubscript{1}\textsuperscript{+} field was optimized over the MRS voxel using the acquired B\textsubscript{1}\textsuperscript{+} maps (37), and an automated local B\textsubscript{0} shim was performed (36). MRS data were acquired in a similar manner as described previously (21). First, eight water spectra (no water suppression or metabolite nulling) were acquired (TE/TR = 38/3700 ms, receiver bandwidth = 4 kHz, voxel volume = 8 cm\textsuperscript{3}) and summed to provide a measure of the total water signal in the voxel. Then, 128 water suppressed full spectra (Figure 4.4a - TE/TR = 38/3700 ms, receiver bandwidth = 4 kHz, voxel volume = 8 cm\textsuperscript{3}) and 128 water suppressed macromolecule spectra (Figure 4.4b - TE/TR = 38/3700 ms, receiver bandwidth = 4 kHz, voxel volume = 8 cm\textsuperscript{3}, T\textsubscript{1}/T\textsubscript{2} = 3.14/0.70s) (metabolite-nulled) were collected in an interleaved manner to minimize subtraction errors due to motion during the acquisition.
Figure 4.4: (a) Representative water suppressed semi-LASER spectrum (TE/TR = 38/3700 ms, 2x2x2 cm$^3$ volume), (b) macromolecule spectrum, (c) HSVD fit of macromolecule spectrum, and (d) resulting metabolite spectrum following macromolecule removal, from one participant (1 Hz exponential filter applied to all spectra shown).

Finally, the transverse decay of the water signal (Figure 4.5) was measured by acquiring water spectra (TR = 5s, receiver bandwidth = 4 kHz, voxel volume = 8 cm$^3$) at different TEs ranging from 38 ms up to 2000 ms. These data were used to calculate the amount of cerebral spinal fluid (CSF) water within the MRS voxel (49-51).
Figure 4.5: Representative plot of water signal (normalized to the signal at the shortest echo time) versus echo time (TE), with superimposed two-component exponential decay fit. The individual contributions from CSF and tissue are shown, illustrating their unique initial amplitudes and decay rates.

The macromolecule spectrum was fitted (Figure 4.4c) using a Hankel singular value decomposition (HSVD, 250 points, 25-35 peaks) (42,52). Although using this fitted curve for subtraction removes the appearance of random noise from the macromolecule spectrum, the underlying uncertainty and error propagates to the metabolite spectrum and decreases the effective signal to noise of this measurement. Since the macromolecule signals are also partially saturated following the inversion pulses designed to null the metabolite signals, the macromolecule spectrum needed to be scaled prior to subtraction (21) from the full spectrum.
The saturation of the macromolecule signal was calculated (using Equation 4.2, $T_{1,\text{MM}} = 430$ ms, $T_{\text{I}_1} = 3.14\text{s}$, and $T_{\text{I}_2} = 0.70\text{s}$) at the time of the acquisition of the full spectrum and then again at the time of the acquisition of the macromolecule spectrum, yielding the macromolecule spectrum scaling factor, 1.64. $T_{1,\text{MM}}$ is the longitudinal relaxation time of the macromolecule signals in the 0.5 - 1.8 ppm range (22). Following scaling, the macromolecule contribution was subtracted from the full spectrum (42,52), yielding the metabolite spectrum (Figure 4.4d).

Prior to fitting the metabolite signals, any remaining unsuppressed water signal was removed by performing a HSVD fit (512 points, auto detected number of peaks) and removing all resonances between 4.2 and 6.2 ppm (Figure 4.6a, water at 4.7 ppm) (9,10,42,43). Fitted metabolite levels were corrected to account for the residual metabolite signal present in the macromolecule spectrum due to incomplete nulling. The contribution of the metabolite to the macromolecule spectrum was estimated using the known metabolite $T_1$ value and the $T_{\text{I}_1}$ and $T_{\text{I}_2}$ values used in the acquisition of the metabolite-nulled spectrum.
Figure 4.6: Representative (a) metabolite spectrum with superimposed fit and (b) the residual difference between the data and fit, from one participant (1 Hz exponential filter applied to the data and the residual). The individual components are shown below the spectrum: (c) NAA, (d) Glu, (e) Gln, (f) GABA, (g) Glth, (h) Asp, (i) Cr, (j) PCr, (k) PC, (l) GPC, (m) Tau, (n) PETH, (o) sI, (p) Glc, and (q) mI.
Metabolite levels were then normalized to the amount of unsuppressed water in each MRS data set and then corrected for T\textsubscript{1} and T\textsubscript{2} relaxation using values determined in this study and from the literature (Table 4.1). The fractional water content was taken as 78%, the average of gray matter and white matter (14). The cerebral spinal fluid (CSF) and tissue (gray matter and white matter) fractions were determined for each \textsuperscript{1}H MRS measurement by fitting the unsuppressed water signal as a function of echo time (TE) to the following bi-exponential decay equation (51):

\[
S(TE) = A_{CSF}e^{-TE/T_{2,CSF}} + A_{tissue}e^{-TE/T_{2,tissue}}
\]

where \(A_{CSF}\) and \(A_{tissue}\) are the initial amplitudes of the CSF and tissue signals, respectively. Fitting was performed using Prism (GraphPad Software, Inc., Version 6.0c, La Jolla, CA). This approach exploits the longer transverse relaxation time constant (\(T_{2} \sim 150\) ms) of CSF water compared to tissue (\(T_{2} \sim 50\) ms) (46) to separate the water decay signal into CSF and tissue components (49-51). Figure 4.5 shows the result of the two-component exponential decay fit in one representative water decay data set.

4.3 Results

4.3.1 Optimal inversion times for metabolite suppression

Water-suppressed spectra with a range of single inversion times were successfully acquired from a 3x3x3 cm\textsuperscript{3} voxel in the parietal-occipital region (Figure 4.1) of all seven
volunteers. The NAA, Glu, tCrCH3, and ChoCH3 peak areas were measured as a function of inversion time (Figure 4.2) and were fitted to the inversion recovery equation (Equation 4.1) to solve for the T1 time constants. The measured T1 time constants for NAA, Glu, tCrCH3, and ChoCH3 are listed in Table 4.1, along with the T1 time constant values of other metabolites taken from the literature. Metabolite T1 time constant values range from ~1.1-2.1s and therefore a single inversion pulse will not null all metabolites simultaneously. To increase nulling efficiency, a double inversion pulse was used to suppress metabolite signals.

To determine the optimal inversion times for the double-inversion nulling scheme, the T1 time constants of NAA, Glu, tCrCH3, and ChoCH3 were each substituted into the double-inversion recovery equation (Equation 4.3) and M2(TI1,TI2) was set equal to zero. This approach yielded four equations that defined the corresponding TI1 and TI2 values that would null a particular metabolite. All four equations were plotted on the same graph (Figure 4.3) and the TI1 and TI2 values that produce optimal simultaneous suppression of all four metabolites were read directly from the plot. These values corresponded to TI1 = 2.09s and TI2 = 0.52s.

4.3.2 Average metabolite concentrations in healthy brain

Figure 4.1 also shows the positioning of the 2x2x2 cm3 1H MRS voxel in the parietal-occipital region of one volunteer. Water-unsuppressed, water-suppressed metabolite, and water-suppressed macromolecule spectra were successfully acquired in all volunteers. The average SNR for the acquired spectra, calculated as the NAA peak intensity over the standard deviation of the baseline, was 48 ± 6. Using the multi-echo transverse decay of the tissue
water signal, two components were successfully identified in each voxel attributed to CSF (long component) and tissue (short component). The average CSF and tissue fractions within the measurement volumes were 8.2 ± 2.0% and 91.8 ± 2.0%, respectively. The macromolecule removal process is demonstrated in one volunteer in Figure 4.4. Figure 4.6 shows a spectrum from one volunteer with: the fit result superimposed onto the data, the residual difference between the data and the fit shown below, and the individual metabolite components shown underneath. Table 4.2 lists the group average and standard deviation of metabolite concentrations in units of mM, as well as the Cramér-Rao lower bounds (53) on quantification precision. The percent coefficient of variation (%CV) was less than 10% for NAA, tCr, Cho, and mI, and less than 20% for Glu and Gln.
Table 4.2: Average metabolite concentrations

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>Average concentration ± SD (mM)</th>
<th>Average Cramér-Rao SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>NAA</td>
<td>11.8 ± 0.8</td>
<td>0.10</td>
</tr>
<tr>
<td>Glu</td>
<td>10.7 ± 1.6</td>
<td>0.15</td>
</tr>
<tr>
<td>Gln</td>
<td>2.2 ± 0.4</td>
<td>0.33</td>
</tr>
<tr>
<td>GABA</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Asp</td>
<td>3.8 ± 1.3</td>
<td>0.08</td>
</tr>
<tr>
<td>NAAG</td>
<td>n.d.</td>
<td>n.d.</td>
</tr>
<tr>
<td>Tau</td>
<td>1.0 ± 0.5</td>
<td>0.09</td>
</tr>
<tr>
<td>Glc</td>
<td>1.2 ± 0.2</td>
<td>0.07</td>
</tr>
<tr>
<td>PEth</td>
<td>1.2 ± 0.5</td>
<td>0.08</td>
</tr>
<tr>
<td>Cr</td>
<td>4.1 ± 0.6</td>
<td>0.36</td>
</tr>
<tr>
<td>PCr</td>
<td>3.5 ± 0.9</td>
<td>0.22</td>
</tr>
<tr>
<td>mI</td>
<td>5.9 ± 0.3</td>
<td>0.05</td>
</tr>
<tr>
<td>Glth</td>
<td>1.6 ± 0.3</td>
<td>0.03</td>
</tr>
<tr>
<td>Scy</td>
<td>0.4 ± 0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>PC</td>
<td>0.3 ± 0.1</td>
<td>0.04</td>
</tr>
<tr>
<td>GPC</td>
<td>1.5 ± 0.4</td>
<td>0.05</td>
</tr>
</tbody>
</table>

SD - standard deviation, n.d. - not detected
4.4 Discussion

The initial goal of this work was to determine the optimal inversion times that would produce a short echo-time metabolite-null macromolecule spectrum for a semi-LASER pulse sequence at 7 Tesla. To this end we measured the in vivo $T_1$ relaxation time constants at 7T for NAA, Glu, tCr$_{CH_3}$, and Cho$_{CH_3}$, which were then used to calculate the inversion times that would maximize metabolite suppression. Subsequently, a subject-specific macromolecule spectrum was acquired alongside each metabolite spectrum using a short-TE single voxel semi-LASER protocol. Following removal of the macromolecule component of the spectrum, the metabolite contributions were fitted with a linear combination of prior knowledge metabolite templates. Using this approach for MRS data collected from the parietal-occipital region of young healthy controls and combining with corrections for tissue partial volume and relaxation, absolute metabolite concentrations were determined by referencing to the unsuppressed water signal within the voxel. Metabolite $T_1$ values (Table 4.1) measured in the current study were consistent with a previous study that also measured in vivo $T_1$ values of metabolites at 7T (22). These values are slightly higher than the $T_1$ values measured at lower field strengths (54,55). Metabolite concentrations were also consistent with previous measures made in a number of different brain regions (Table 4.3).

The semi-LASER (33-35) MRS sequence used for volume selection in the current study, utilized a 90° sinc pulse for slice selection, and 2 pairs of AFP pulses to refocus the signal in the remaining two orthogonal planes. Adiabatic pulses have excellent slice-selection profiles (26) compared to conventional pulses used in STEAM and PRESS, reducing contamination from outside of the measurement volume. Therefore unlike in the cases of
STEAM and PRESS, OVS was not required in the current study. The semi-LASER sequence was used instead of the conventional LASER sequence (26) to reduce the number of high-power AFP pulses from 6 to 4, resulting in lower power deposition and a shorter minimum TE.
Table 4.3: Reported metabolite concentrations measured with 7T single-voxel $^1$H MRS

<table>
<thead>
<tr>
<th>Study</th>
<th>N</th>
<th>Sequence</th>
<th>Brain region</th>
<th>SNR</th>
<th>NAA</th>
<th>Glu</th>
<th>Gln</th>
<th>tCr</th>
<th>Cho</th>
<th>mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>[Ref] [year]</td>
<td></td>
<td>[Age] [TE]</td>
<td>[NT]</td>
<td>[Volume]</td>
<td>48±6</td>
<td>11.8</td>
<td>10.7</td>
<td>2.2</td>
<td>7.6</td>
<td>1.8</td>
</tr>
<tr>
<td>Current study</td>
<td>5</td>
<td>semi-LASER POC</td>
<td>38 ms</td>
<td>8 mL</td>
<td>128</td>
<td>0.8</td>
<td>1.6</td>
<td>0.4</td>
<td>0.4</td>
<td>0.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%SNR</td>
<td>6.7</td>
<td>15</td>
<td>20</td>
<td>5.9</td>
<td>8.5</td>
</tr>
<tr>
<td>Wijten.</td>
<td>4</td>
<td>STEAM</td>
<td>AC</td>
<td>47.5</td>
<td>Mean^†</td>
<td>12.8</td>
<td>15.5</td>
<td>3.7</td>
<td>9.1</td>
<td>2.3</td>
</tr>
<tr>
<td>(32)</td>
<td>24±2</td>
<td>14 ms</td>
<td>32</td>
<td>38 mL</td>
<td>St.D.</td>
<td>1.2</td>
<td>0.4</td>
<td>0.6</td>
<td>0.7</td>
<td>0.2</td>
</tr>
<tr>
<td>2013</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%SNR</td>
<td>9.4</td>
<td>2.6</td>
<td>16</td>
<td>7.7</td>
<td>8.7</td>
</tr>
<tr>
<td>DLPFC</td>
<td>46</td>
<td>Mean^†</td>
<td>10.5</td>
<td>10.1</td>
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<td>4.7</td>
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<tr>
<td></td>
<td></td>
<td>St.D.</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>0.6</td>
<td>0.2</td>
<td>0.9</td>
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<tr>
<td></td>
<td></td>
<td>%SNR</td>
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<td>12</td>
<td>35</td>
<td>9.2</td>
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<td>19</td>
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<tr>
<td>Steph.</td>
<td>12</td>
<td>STEAM</td>
<td>ACC</td>
<td>63±10</td>
<td>Mean^†</td>
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<td>2.3</td>
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<td>1.6</td>
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<tr>
<td>(30)</td>
<td>28±11</td>
<td>16 ms</td>
<td>288</td>
<td>16 mL</td>
<td>St.D.</td>
<td>0.7</td>
<td>1.4</td>
<td>0.4</td>
<td>0.6</td>
<td>0.2</td>
</tr>
<tr>
<td>2011</td>
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<td></td>
<td>%SNR</td>
<td>11</td>
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<td>17</td>
<td>10</td>
<td>13</td>
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<tr>
<td></td>
<td></td>
<td>Insula</td>
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<td>Mean^†</td>
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<td>12.1</td>
<td>2.5</td>
<td>6.5</td>
<td>1.7</td>
<td>3.8</td>
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<td></td>
<td></td>
<td>St.D.</td>
<td>0.6</td>
<td>1.3</td>
<td>0.5</td>
<td>0.4</td>
<td>0.2</td>
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<tr>
<td></td>
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<td>%SNR</td>
<td>8.5</td>
<td>11</td>
<td>20</td>
<td>6.2</td>
<td>12</td>
<td>13</td>
<td></td>
<td></td>
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<tr>
<td>Mekle</td>
<td>6</td>
<td>SPECIAL</td>
<td>OC</td>
<td>77±5</td>
<td>Mean^*</td>
<td>11.8</td>
<td>9.9</td>
<td>2.2</td>
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<td>1.1</td>
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<tr>
<td>(15)</td>
<td>22-26</td>
<td>6 ms</td>
<td>64</td>
<td>22 mL</td>
<td>St.D.</td>
<td>0.2</td>
<td>0.9</td>
<td>0.4</td>
<td>0.4</td>
<td>0.05</td>
</tr>
<tr>
<td>2009</td>
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<td></td>
<td></td>
<td></td>
<td>%SNR</td>
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<td>9.1</td>
<td>18</td>
<td>5.0</td>
<td>4.5</td>
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<tr>
<td>Tkac</td>
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<td>OC</td>
<td>194±27</td>
<td>Mean^*12.1</td>
<td>9.6</td>
<td>2.8</td>
<td>8.4</td>
<td>1.2</td>
<td>6.3</td>
</tr>
<tr>
<td>(31)</td>
<td>24±5</td>
<td>6 ms</td>
<td>160</td>
<td>8 mL</td>
<td>St.D.</td>
<td>0.7</td>
<td>0.6</td>
<td>0.4</td>
<td>0.6</td>
<td>0.1</td>
</tr>
<tr>
<td>2009</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>%SNR</td>
<td>5.8</td>
<td>6.3</td>
<td>14</td>
<td>7.1</td>
<td>8.3</td>
</tr>
</tbody>
</table>

N - number of participants, NT - number of averages, semi-LASER - semi-localization by adiabatic selective refocusing, POC - parietal-occipital cortex, %CV - percent coefficient of variance, STEAM - stimulated echo acquisition mode, AC - anterior cingulate, DLPFC - dorsolateral prefrontal cortex, ACC - anterior cingulate cortex, SPECIAL - spin-echo full-intensity acquired localized, OC - occipital cortex

* - mM concentration

^† - institutional units of concentration

%CVs from previous studies were calculated from the reported mean and standard deviations - (St. Dev/Mean) X 100, and mean and standard deviations for Tkac et. al., 2009 were read from bar graphs as they were not listed explicitly.
The macromolecule contribution to the short-TE spectrum was measured for each participant. Although this approach adds ~8 min/scan, it ensures that the macromolecule signals in each spectrum are accurately represented rather than approximated using a generalized macromolecule lineshape in the prior knowledge template. Furthermore, the full and macromolecule spectra were acquired in an interleaved fashion (21) to minimize subtraction errors due to subject movement during the scan (~16 min to acquire the full and macromolecule spectra). The macromolecule spectrum was acquired by nulling the metabolite signals using a double-inversion preparation (38), as macromolecules and metabolites have largely different T1’s (~430 ms for macromolecules and ~1600 ms for metabolites at 7T) (22). The inversion times used to acquire the macromolecule spectrum depend on the T1 time constants of the metabolites that are to be nulled. However effective nulling can be achieved across a large range of inversion times as shown in Figure 4.3. In the current study, the macromolecule spectra were acquired with TI1/TI2 = 3.14/.070s, which was somewhat different than the predicted optimal TI1/TI2 = 2.09/0.52s. However using these inversion times the macromolecule signals were effectively removed as demonstrated in Figure 4.4. The residual signals in the metabolite spectrum between 0.5 - 1.8 ppm are likely from macromolecule components that are not completely removed following subtraction of the macromolecule spectrum. The most likely reason of the incomplete removal is that the macromolecule T1’s vary as previously shown by de Graaf et. al. (45). The residual metabolite signals observed in the macromolecule spectrum, most notably NAA at 2.01 ppm and tCr at 3.93 ppm, (Figure 4.4b and Figure 4.4c) could be reduced in future experiments by using TI1/TI2 = 2.09/0.52s. However, the error in metabolite levels due to residual metabolite
signals in the macromolecule spectrum was estimated for each metabolite by substituting the measured $T_1$s (Table 4.1) along with $T_1 = 3.14\text{s}$ and $T_2 = 0.70\text{s}$ into Equation 4.2. The calculated contributions of each metabolite to the macromolecule spectrum ranged from 0.6% to 16% of the metabolite area. This contribution was used to correct the absolute metabolite concentration measurements, listed in Tables 4.2 and 4.3. In a previous study by our group using a LASER pulse sequence at 4T with an echo time of 46 ms, the maximum error in metabolite levels due to residual metabolite signals in the macromolecule spectrum was calculated to be 6% (21).

The concentrations and coefficients of variance (CV) of NAA, Glu, Gln, tCr, Cho, and mI from this study were compared to previous 7T studies that reported metabolite concentrations in healthy brain (15,30-32), summarized in Table 4.3. Of note, all four of the previous studies at 7T used LCModel (8,56) for fitting with prior knowledge about metabolites and macromolecule resonances, while the current study acquired subject-specific macromolecule information. Also, all four of those studies corrected for water content using water fractions within LCModel based on $T_1$-weighted images, while the current study exploited differences in the tissue and CSF water $T_2$ relaxation times to determine the CSF and tissue water content within the measurement volume (51). Nonetheless, the coefficients of variance for the metabolite measurements in the current study were comparable to the previous studies. The average levels of NAA, Glu, Gln, tCr, Cho, and mI were all within ~1 mM of the two studies that reported mM levels (15,31). Finally, the average SNR in this study (48 ± 6) is comparable to the average SNR in four out of five regions in the previous studies (ranging from 36 to 77), while one study reported an average SNR of 194 ± 27 (31).
These results indicate that this 7T short-TE semi-LASER $^1$H MRS protocol, incorporating subject-specific macromolecule removal, yields accurate and reproducible metabolite concentrations in human brain consistent with previous results.

Although measuring metabolite levels within the parietal-occipital cortex allowed for comparison with previous studies at 7T, future studies would benefit from measuring metabolite levels and their coefficients of variance from multiple brain regions including the hippocampus. Further, the measured $T_1$ values and therefore metabolite levels could have been effected by the direct saturation of the water signal due to magnetization transfer effects between water and metabolite resonances via chemical exchange (57). Therefore metabolite quantification methods that do not suppress the water signal may be worth investigating in future studies.

### 4.5 Conclusions

A short TE $^1$H semi-LASER spectroscopy protocol incorporating subject-specific macromolecule removal was developed to quantify absolute metabolite levels in the human brain. The optimal double inversion delay times for metabolite suppression leading to macromolecule detection were $TI_1 = 2.09s$ and $TI_2 = 0.52s$. Metabolite concentrations measured in the parietal-occipital region of young healthy volunteers were in good agreement with previous studies performed in healthy brain at 7T. Measurement reproducibility was also similar to previous work. Incorporating subject specific macromolecule measurement into metabolite quantification is important in the application of short echo-time spectroscopy
to pathological conditions such as cancer, where macromolecule concentrations may deviate from the norm.

4.6 Acknowledgements

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4.7 References


Chapter 5

Summary and future work

This chapter includes a summary of the main scientific contributions, and suggests areas that require further investigation and development.

5.1 Summary

5.1.1 Increased glutamate in the hippocampus after galantamine treatment for Alzheimer disease

Chapter 2 describes a study where short echo time $^1$H magnetic resonance spectroscopy at 4 Tesla was used to measure metabolite concentrations within the right hippocampus of ten subjects with AD following four months of galantamine treatment. The absolute glutamate (Glu) concentration increased, as did the ratio of Glu to total creatine (tCr) and the ratio of Glu to N-acetylaspartate (NAA), while the Mini-Mental State Examination (MMSE) and the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) scores did not change significantly. The change in the Glu/tCr ratio was positively correlated with the change in the MMSE scores and the change in the ratio of Glu to myo-inositol (mI) was inversely correlated with the change in the ADAS-cog scores, both suggesting that these metabolite
ratios are related to cognitive function. The increase in glutamate observed may be related to the action of galantamine as an allosteric potentiating ligand for presynaptic nicotinic acetylcholine receptors, which increases glutamatergic neurotransmission.

5.1.2 Reduced $N$-acetylaspartate to creatine ratio in the posterior cingulate correlates with cognition in Alzheimer disease following four months of rivastigmine treatment

Chapter 3 describes a study where long echo time $^1$H magnetic resonance spectroscopy at 3 Tesla was used to measure metabolite concentrations within bilateral posterior cingulate cortex of fourteen subjects with AD following four months of rivastigmine treatment. The ratio of $N$-acetylaspartate (NAA) to total creatine ($t$Cr) decreased by 12.7% following four months of rivastigmine treatment, while no other metabolite ratio changed and no absolute metabolite concentration changed. Further, neither the Mini-Mental State Examination (MMSE) scores or the Alzheimer Disease Assessment Scale-cognitive subscale (ADAS-cog) scores changed. However, the change in the ratio of NAA/$t$Cr correlated with the change in MMSE scores. This association between changes in NAA/$t$Cr and cognitive performance suggest that NAA/$t$Cr could be an objective indicator of response to rivastigmine, as a symptomatic treatment. Further, the decrease in NAA/$t$Cr suggests continued neuronal impairment within the posterior cingulate in AD during rivastigmine treatment. The association between changes in NAA/$t$Cr and cognitive performance suggest that NAA/$t$Cr could be an objective indicator of response to rivastigmine treatment.
5.1.3 Semi-LASER $^1$H MR spectroscopy at 7 Tesla in human brain: metabolite quantification incorporating subject-specific macromolecule removal

Chapter 4 details the development of a short echo-time $^1$H semi-LASER spectroscopy protocol incorporating subject-specific macromolecule removal to quantify absolute metabolite levels in the human brain at 7 Tesla. Metabolite levels were measured in the parietal-occipital region of young healthy volunteers, where the average signal to noise ratio, $N$-acetylaspartate (NAA) peak height divided by the baseline noise standard deviation, was $48 \pm 6$. The $T_1$ time constants for NAA, glutamate (Glu), total creatine (tCr), and choline (Cho) were $1.71 \pm 0.15s$, $1.68 \pm 0.19s$, $1.63 \pm 0.10s$, and $1.41 \pm 0.09s$, respectively. The optimal double inversion delay times for metabolite suppression leading to macromolecule detection were $TI_1 = 2.09s$ and $TI_2 = 0.52s$. The coefficient of variation (COV) was less than 10% for NAA, tCr, Cho, and myo-inositol (mI), and less than 20% for Glu and glutamine (Gln). Metabolite concentrations and COVs were in agreement with previous studies performed in healthy brain at 7 Tesla, as was measurement reproducibility. Short-echo-time $^1$H semi-LASER spectroscopy at 7T incorporating subject-specific macromolecule removal yielded reproducible brain metabolite concentrations ideal for applications in disease conditions where macromolecule contributions may deviate from the norm.
5.1.4 Conclusions

The first aim of this thesis work was to investigate potential metabolic biomarkers of Alzheimer disease (AD) that would improve diagnosis and sensitivity to subtle disease changes. Proton magnetic resonance spectroscopy ($^{1}$H MRS) was used to measure metabolic changes following cholinesterase inhibitor treatment. In one $^{1}$H MRS study at 4 Tesla (Chapter 2), an increase in the excitatory neurotransmitter glutamate was detected in the right hippocampus and was associated with increased cognitive performance. In a second $^{1}$H MRS study at 3 Tesla (Chapter 3), the ratio of the neuronal marker $N$-acetylaspartate to total creatine was decreased in the bilateral posterior cingulate cortex, which was associated with cognition. The fact that both of these studies detected metabolic changes that correlated with cognition without significant cognitive changes may suggest that $^{1}$H MRS is more sensitive to changes.

The second aim of this thesis work was to develop a 7 Tesla $^{1}$H MRS data acquisition and metabolite quantification protocol to be used for future human neurological studies (Chapter 4). This protocol incorporates subject-specific macromolecule removal and yielded absolute in vivo metabolite concentrations that are in agreement with previous studies, making it ideal for applications in disease conditions where macromolecule contributions may deviate from the norm.
5.2 Future Work

5.2.1 Proton MR spectroscopy of Alzheimer disease

Measuring metabolic fluctuations in regions of the brain that are affected in Alzheimer disease (AD) is logical when studying the disease, but doing so in multiple regions in the same study would be beneficial (i.e. in the hippocampus and posterior cingulate). This could allow one to determine more precisely what metabolites fluctuate in what regions, and if different affected regions behave similarly. It would also be beneficial to study a region of the brain that is not as affected by AD, such as the cerebellum, as a control region. This internal control could help eliminate outliers and could negate the need for a control cohort. However, acquiring multiple single-voxel data sets is difficult due to increased scan time, and therefore more patient discomfort particularly in elderly subjects.

Additionally, a control cohort of untreated AD patients would benefit treatment studies but as recommended at the Third Canadian Consensus Conference on Diagnosis and Treatment of Dementia (3rd CCCDTD, 2006), “provision of the best standard of care for the patient must always remain the priority” (1), and therefore ethics must be considered when denying a patient the standard AD treatment.

The relatively short treatment durations (four months) used in the two AD treatment studies could have benefited from additional follow-up measurement time-points. It would be interesting to know if the changes in metabolite levels detected after four months would continue or if they level off. It would also be interesting to know if at a later time-point the cognitive measures would detect differences.

$^1$H MRS will remain a useful and relevant tool for neuroimaging researchers due to its
unique ability to non-invasively detect a number of metabolic by-products in aging and disease, allowing for the evaluation of medications that aim to alter brain biochemistry. However, improved inter-site methodological and data quality standards would allow for better comparison between studies and research groups.

5.2.2 Proton MR spectroscopy protocol at 7 Tesla

Although measuring metabolite levels within the parietal-occipital cortex allowed for comparison with previous studies at 7 Tesla, future studies would benefit from measuring metabolite levels and their coefficients of variance from multiple brain regions including more demanding regions such as the hippocampus. Further, it would be beneficial to measure metabolite levels in an older population to act as a control cohort of future disease and treatment studies.

The water signal decay versus echo time fitting method served well to determine the voxel cerebral spinal fluid (CSF) and tissue (grey matter and white matter) fractions. However, it may benefit future studies to incorporate the more commonly used image segmentation method into the 7 Tesla $^1$H MRS protocol detailed in Chapter 4. This would allow for a more detailed grey matter, white matter, CSF voxel segmentation and potentially more accurate partial volume and signal relaxation corrections.

Lastly, metabolite quantification methods that do not suppress the water signal may be worth investigating to avoid possible magnetization transfer effects between water and metabolite resonances via chemical exchange (2).
5.3 References


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Appendix B

Ethics Approval
B.1 Ethics approval for human Alzheimer’s MRS experiments

Office of Research Ethics
The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 3K1
Telephone: (519) 855-5356 Fax: (519) 855-2486 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. M.J. Borrie
Review Number: 06673E
Review Date: November 30, 2009
Review Level: Expedited
Protocol Title: Magnetic resonance spectroscopy as a measurement of response to cholinergic treatment in Alzheimer’s disease (Pilot study)
Department and Institution: Neurology, Parkinson Hospital
Sponsor: LAVISION HEALTH RESEARCH INSTITUTE
Ethics Approval Date: November 30, 2009
Expiry Date: December 31, 2010
Documents Reviewed and Approved: Revised study methodology, revised protocol, revised consent form (Version 12, November 2009).

Documents Received for information:

This is to notify you that The University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) has reviewed and approved According to the Tri-Council Policy Statement: Ethical Conduct for Research Involving Humans and the Health Canada/ICCH Good Clinical Practice Practice: Consolidated Guidelines, and the applicable laws and regulations of Western has approved into the above referenced revision(s) or amendment(s) to the approval date noted above. The membership of this REB also complies with the membership requirements for REB’s as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time you must request one using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the change(s) involve only logistical or administrative aspects of the study (e.g., change of investigator, telephone number). Expedited review of minor change(s) in ongoing studies will be considered. Subjects must receive a copy of this notification. Consent documentation.

Investigators must promptly also report to the HSREB:

a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
b) all adverse or unexpected experiences or events that are both serious and unanticipated;
c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these adverse events require a change to the information/consent documentation, and/or recruitment or advertising, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, or vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gioia

Ethics Officer to Contact for Further Information

[Signature: ]
[Signature: ]
[Signature: ]
[Signature: ]

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UWO HSREB Ethics Approval - Revision
V2008-07-17 (approved by the HSREB: [signature] [date])
06673E
Page 1 of 1
B.2 Ethics approval for human 7T development study

Office of Research Ethics
The University of Western Ontario
Room 4180, Support Services Building, London, ON, Canada N6A 3G1
Telephone: (519) 661-3036 Fax: (519) 661-2485 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.S. Gall
Review Number: 13018
Review Date: August 21, 2008

Protocol Title: 7 Tesla MRI Hardware and Software Development
Department and Institution: Imaging, Roberta Research Institute
Sponsor:

Ethics Approval Date: October 02, 2008
Expiry Date: July 31, 2018

Documents Reviewed and Approved:
Revised study methodology, revised sample size, revised protocol and revised Letter of Information and Consent Form version 1.2

Documents Received for Information:

This is to notify you that the University of Western Ontario Research Ethics Board for Health Sciences Research involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement: Ethical Conduct of Research Involving Human Subjects, has reviewed the proposal and granted approval to the above referenced revision(s) or amendment(s) to the original approval dated above. The membership of this REB also complies with the membership requirements for REB's as defined in Division 6 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time, you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the patient(s) involve only logistical or administrative aspects of the study (e.g., change of monitor, telephone number). Expedited review of minor changes in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly report to the HSREB:

a) any changes in the risk to the participant(s) and/or affecting significantly the conduct of the study;

b) all adverse and unexpected experiences or events that are both serious and unexpected;

c) any information that may adversely affect the safety of the subject or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussions related to, nor review, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert

Ethics Officer to Contact for Further Information:

Dr. Elizabeth Wambolt (ewambolt@uwo.ca)

Dr. Grace Kelly (grace.kelly@uwo.ca)

Dr. Denise Crafton (dcrafton@uwo.ca)

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Office of Research Ethics
The University of Western Ontario
Room 4180 Support Services Building, London, ON, Canada N6A 6C1
Telephone: (519) 661-3036 Fax: (519) 852-2466 Email: ethics@uwo.ca
Website: www.uwo.ca/research/ethics

Use of Human Subjects - Ethics Approval Notice

Principal Investigator: Dr. J.S. Gati
Review Number: 15018
Review Date: March 31, 2010

Protocol Title: 7 Tesla MRI Hardware and Software Development
Department and Institution: Imaging, Roberta Research Institute
Sponsor:

Ethics Approval Date: March 31, 2010 Expiry Date: July 31, 2018
Documents Reviewed and Approved: Administrative Changes: Study Personnel
Documents Received for Information:

This is to notify you that the University of Western Ontario Research Ethics Board for Health Sciences Research Involving Human Subjects (HSREB) which is organized and operates according to the Tri-Council Policy Statement, Ethical Conduct of Research Involving Human Subjects and the Health Canada/ICH Good Clinical Practice (GCP) Guidelines, and the applicable laws and regulations of Ontario has reviewed and granted approval to the above referenced revision(s) or amendment(s) on the approval date noted above. The membership of this HSREB also complies with the membership requirements for REB's as defined in Division 5 of the Food and Drug Regulations.

The ethics approval for this study shall remain valid until the expiry date noted above assuming timely and acceptable responses to the HSREB's periodic requests for surveillance and monitoring information. If you require an updated approval notice prior to that time, you must request it using the UWO Updated Approval Request Form.

During the course of the research, no deviations from, or changes to, the Protocol or consent form may be initiated without prior written approval from the HSREB except when necessary to eliminate immediate hazards to the subject or when the changes involve only logistical or administrative aspects of the study (e.g., change of monitor, telephone number). Expedited review of minor changes(s) in ongoing studies will be considered. Subjects must receive a copy of the signed information/consent documentation.

Investigators must promptly report to the HSREB:

- a) changes increasing the risk to the participant(s) and/or affecting significantly the conduct of the study;
- b) all adverse and unexpected experiences or events that are both serious and unexpected;
- c) new information that may adversely affect the safety of the subjects or the conduct of the study.

If these changes/adverse events require a change to the information/consent documentation, and/or recruitment advertisement, the newly revised information/consent documentation, and/or advertisement, must be submitted to this office for approval.

Members of the HSREB who are named as investigators in research studies, or declare a conflict of interest, do not participate in discussion related to, nor vote on, such studies when they are presented to the HSREB.

Chair of HSREB: Dr. Joseph Gilbert
F2H Ref #: PICD 60006940

Ethics Officer to Contact for Further Information

Janice Sunderland (jsunder@uwo.ca) Elizabeth Warnbot (ewarnbot@uwo.ca) Grace Kelly (grace.kelly@uwo.ca) Denise Grattan (dgrattan@uwo.ca)

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UWO HSREB Ethics Approval - Revision:
V.2002-07-07 15018(Version=0) 15018 15018 Page 1 of 1
Curriculum Vitae
Jacob Penner
As of August 2014

Department Address
Centre for Functional and Metabolic Mapping
Robarts Research Institute, Western Ontario
100 Perth Drive, London, ON, Canada, N6A 5K8

Education

2007 - Present
PhD Candidate: Department of Medical Biophysics, Western University
Reclassified from MSc in April 2009.
Thesis: Magnetic Resonance Spectroscopy of Alzheimer Disease
Supervisor: Dr. Robert Bartha

2002 - 2007
BSc: Department of Medical Physics and Radiation Sciences
McMaster University
Concentration: Honours Medical and Health Physics

Employment History

I analyzed computed tomography (CT) images of mice to track tumour growth.
Supervisor: Ting-Yim Lee, PhD

May 2007 - Aug 2007 Research Student, Centre for Functional and Metabolic Mapping,
Robarts Research Institute, London, Ontario
I performed Magnetic Resonance Imaging and Spectroscopy scans on subjects
with Alzheimer disease, and analyzed the spectroscopic data.
Supervisor: Rob Bartha, PhD

May 2006 - Aug 2006 Research Student, Department of Medical Physics and Radiation Sciences,
McMaster University, Hamilton, Ontario
I designed, built, and performed neutron activation analysis on aluminum/brain
phantoms in an Alzheimer’s disease study.
Supervisor: Fiona McNeill, PhD

Oct 2004 - Aug 2005 Co-operative Education Student, Juravinski Cancer Centre, Hamilton, Ontario
I assisted in the design and manufacturing of an optical imaging system, and
subsequently imaged cancer tumours in mice.
Supervisor: Aram Kudian, PhD
May 2004 - Aug 2004  | Research Student, Department of Medical Physics and Radiation Sciences, McMaster University, Hamilton, Ontario
I found the detection limit of indium in lung phantoms using neutron activation analysis.
Supervisor: Fiona McNeill, PhD

May 2003 - Aug 2003  | Research Student, Department of Chemistry, McMaster University, Hamilton, Ontario
I imaged functionalized carbon nanotubes with a scanning tunneling microscope.
Supervisor: Peter Kruse, PhD

Awards

<table>
<thead>
<tr>
<th>Date</th>
<th>Award Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>May 2011</td>
<td>OGS - Ontario Graduate Scholarship, $15,000</td>
</tr>
<tr>
<td>April 2012</td>
<td>A provincial award based on academic and research achievements.</td>
</tr>
<tr>
<td>Sept 2010</td>
<td>OGSST - Ontario Graduate Scholarship in Science &amp; Technology, $10,000</td>
</tr>
<tr>
<td>April 2011</td>
<td>A provincial award based on academic and research achievements.</td>
</tr>
<tr>
<td>Sept 2009</td>
<td>OGS - Ontario Graduate Scholarship, $15,000</td>
</tr>
<tr>
<td>Aug 2010</td>
<td>A provincial award based on academic and research achievements.</td>
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<td>May 2008</td>
<td>OGSST - Ontario Graduate Scholarship in Science &amp; Technology, $15,000</td>
</tr>
<tr>
<td>April 2009</td>
<td>A provincial award based on academic and research achievements.</td>
</tr>
<tr>
<td>June 2008</td>
<td>London Imaging Discovery Conference first place poster prize, $300</td>
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<tr>
<td></td>
<td>An award given to the best research poster.</td>
</tr>
<tr>
<td>Sept 2007</td>
<td>Western Graduate Research Scholarship, ~$6,600/year for 5 years</td>
</tr>
<tr>
<td>Aug 2012</td>
<td>Awarded for maintaining a grade point average over 80%.</td>
</tr>
<tr>
<td>Sept 2005</td>
<td>The Emanuel Williams Scholarship in Physics, McMaster University, $800</td>
</tr>
<tr>
<td></td>
<td>Awarded to the Physics student with the highest second-year course average.</td>
</tr>
<tr>
<td>Sept 2004</td>
<td>The University Senate Scholarship, McMaster University, $800</td>
</tr>
<tr>
<td>April 2005</td>
<td>An award based on academic achievement of the first two years of my undergraduate degree.</td>
</tr>
<tr>
<td>May 2004</td>
<td>NSERC Undergraduate Summer Fellowship, $6,200</td>
</tr>
<tr>
<td>Aug 2004</td>
<td>A national award based on academic achievement over the first two years of my undergraduate degree.</td>
</tr>
<tr>
<td>Sept 2003</td>
<td>The Bill Prestwich Scholarship in Medical and Health Physics, $500</td>
</tr>
<tr>
<td></td>
<td>Awarded to the student entering the Honours Medical and Health Physics program at McMaster University with the highest first-year course average.</td>
</tr>
</tbody>
</table>
May 2003 - Aug 2003  Chemistry Summer Research Scholarship, McMaster University, $5,600
Awarded to the applicant with the highest first-year course average.

Sept 2002 - April 2006  Ontario Aiming for the Top Tuition Scholarship, $3,472/year for three years
Awarded for maintaining a grade point average over 80%.

2002 - 2003  The McMaster Honour Award, $2,000/year for two years
Awarded to students entering university who maintained a high school course average over 90%.

Publications

4. Jacob Penner, Jennie L. Wells, Michael J. Borrie, Sarah M. Woolmore-Goodwin, Robert Bartha. Reduced N-acetylaspartate to creatine ratio in the posterior cingulate correlates with cognition in Alzheimer disease following four months of rivastigmine treatment. Dement Geriatr Cogn Disord. Accepted August 19, 2014.


Conference Presentations


11. **Jacob Penner**, Matthew Smith, Jennie Wells, Michael Borrie, Robert Bartha. 3T Magnetic Resonance Spectroscopy of the Posterior Cingulate Following Rivastigmine Treatment in Alzheimer Disease. Poster Presentation, Canadian Conference on Dementia, Montreal, Quebec, Canada: October 27-29, 2011.


