Neurotransmitter Imaging of the Human Brain

Detecting γ -Aminobutyric Acid (GABA) Using
Magnetic Resonance Spectroscopy

Sofie Tapper

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Cover picture: An abstract illustration of a GABA-edited difference spectrum.

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KEEP CALM AND BE LIKE A PANDA!

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ABSTRACT

Introduction: In this thesis, MEGA-edited Magnetic Resonance Spectroscopy (MRS) has been used for the purpose of non-invasive detection of γ -aminobutyric acid (GABA) within the brain. GABA is the main inhibitory neurotransmitter in the human central nervous system, and glutamate is the corresponding main excitatory neurotransmitter. A balance between GABA and glutamate is crucial for healthy neurotransmission within the brain, and regional altered concentrations have been linked to certain neurological disorders. However, it is challenging to measure GABA, and special editing approaches are needed in order to allow reliable quantification. In addition, the GABA measurement is further complicated due to disturbances such as movements during the acquisition that may lead to artifacts in the resulting spectrum.

This thesis can be divided into two sections, where the first section focuses on three clinical applications (narcolepsy, irritable bowel syndrome (IBS), and essential tremor (ET)), which were all investigated using MEGA-edited single-voxel spectroscopy (SVS). The second section focuses on method development, where two statistical retrospective approaches were investigated for the purpose of improving MEGA-edited data. In addition, a new MRS imaging (MRSI) pulse sequence with the purpose of GABA detection using a high spatial resolution, short acquisition time, and full brain coverage was also investigated.

Materials and Methods: In total, 164 participants were included and 272 MRS measurements were performed with the voxel placed in the medial prefrontal cortex (mPFC, 136), thalamus (32), and cerebellum (104) using two different but “identical” MR systems. Nineteen narcolepsy patients and 21 matched healthy controls performed an fMRI working memory task using a simultaneous EEG followed by an mPFC GABA-edited MRS measurement. Sixty-four IBS patients and 32 matched healthy controls underwent an mPFC GABA-edited MRS measurement followed by resting state fMRI. In addition, psychological symptoms were assessed using questionnaires. Ten ET patients and six matched healthy controls underwent four GABA-edited MRS measurements with the voxels placed in the thalamus and cerebellum. In this study, the symptom severity was investigated using the essential tremor rating scale (ETRS). All clinical MRS datasets were analyzed using conventional methods for post-processing and quantification. Furthermore, 12 volunteers were recruited for the purpose of investigating statistical retrospective approaches for artifact detection and elimination of MRS data. Each participant underwent three reference measurements and three measurements with induced head

movements conducted according to a movement paradigm. Order statistic filtering (OSF) and jackknife correlation (JKC) were investigated as regards to the elimination of artifact-influenced spectra and reliability of the resulting concentrations. Finally, phantom measurements were performed for the purpose of investigating MEGA-edited MRSI.

Results: In narcolepsy, a trend-level association was observed between the mPFC MRS concentrations and increased deactivation within the default mode network during the working memory task. A significantly higher mPFC GABA+ concentration was observed in IBS patients with a high severity of comorbid anxiety. In ET, a positive correlation was observed between cerebellar GABA+/Glx ratios and tremor severity. Moreover, movements during the measurement decreased the concentration estimates due to signal loss in the spectra. Both OSF and JKC resulted in trend-level improvement of the signal-intense metabolites in spectrum when artifacts were present in the data, while performing equally as well as conventional analysis methodology when no intentional movements were present in the data. Finally, using the fast MEGA-edited multi-voxel sequence developed for a conventional clinical scanner, our phantom measurements showed that GABA was detectable using a 1:45 min acquisition time and an MRSI voxel size of 1 mL.

Discussion: Several challenges such as time constraints, large voxel sizes, and protocol design were encountered when performing SVS MEGA-PRESS in the clinical research settings. In addition, artifacts in the MRS data originating for example, from motions, negatively impacted the resulting averaged spectra, which was evident in both data from clinical populations and healthy controls. In the presence of artifacts in the data, both OSF and JKC improved the SVS MEGA-edited spectra. In addition, the implemented JKC method can be used not only for artifact detection, but also as a generally applicable retrospective technique for the quality control of a dataset, or as an indication of whether a shift in voxel placement occurred during the measurement.

Using the MEGA-edited MRSI pulse sequence, there are many technical challenges, including detrimental effects from eddy currents, spurious echoes, and field inhomogeneities. Even though there are many technical challenges when using MEGA-edited MRSI, an optimized version of the MRSI sequence would be extremely valuable in clinical research applications where high spatial resolution and short acquisition times are highly desired.

Conclusions: OSF and JKC improved the metabolite quantification when artifacts were present in the data, and JKC was preferable. Although there are many technical challenges, MEGA-edited MRSI with full brain coverage in combination with a minimal voxel size for the purpose of GABA detection, would be extremely useful in clinical research applications where disorders such as narcolepsy, IBS, or ET, are investigated.

SAMMANFATTNING

I denna avhandling har MEGA-editerad magnetresonansspektroskopi (MRS) använts med syfte för icke-invasiv detektion av GABA i hjärnan. GABA, eller gamma-aminosmörtsyra, är den vanligaste hämmande signalsubstansen i det centrala nervsystemet, och glutamat är den vanligaste motsvarande excitatoriska signalsubstansen. GABA och glutamat ska existera i balans i den friska hjärnan, och många studier har visat samband mellan regionala förändringar i koncentrationerna av signalsubstanser och vissa neurologiska sjukdomar. Dock är det en utmaning att mäta GABA, och speciella editeringsmetoder (MEGA) behövs för tillförlitlig kvantifiering. Dessutom blir GABA-mätningen ännu mer komplicerad då störningar från exempelvis rörelse under mätningen som kan ge artefakter i det resulterande spektrumet.

Denna avhandling kan delas upp i två delar, där första delen behandlar tre kliniska forskningsapplikationer av single-voxel MEGA-editerad spektroskopi (SVS) där patienter diagnostiserade med narkolepsi, IBS, och essentiell tremor (ET) undersökts. Den andra delen omfattar metodutveckling, där först två retrospektiva tekniker för detektion och eliminering av spektrum med dålig kvalitet undersökts. Slutligen utforskades även en ny pulssekvens implementerad för multi-voxel MEGA-editerad MRS (MRSI), med syfte för snabb högupplöst GABA detektion som täcker hela hjärnan.

Totalt deltog 164 patienter och frivilliga i de olika studierna i denna avhandling, och totalt utfördes 272 MRS-mätningar med voxeln placerad i tre olika regioner i hjärnan. I narkolepsistudien rekryterades 19 patienter och 21 matchade friska frivilliga, som undersöktes med funktionell MRI och EEG samtidigt som de genomförde en uppgift med syfte att testa arbetsminnet, och därefter mättes GABA frontalt i hjärnan. I IBS-studien undersöktes 64 patienter och 32 friska frivilliga med frontal MRS som följdes av funktionell MRI. Dessutom utvärderades komorbida psykologiska symtom med frågeformulär. I ET-studien undersöktes 10 patienter och 6 friska frivilliga med voxlar placerade i talamus och i lillhjärnan. Graden av tremor och besvär utvärderades med ETRS. Alla kliniska MRS mätningar analyserades med konventionell analysmetod.

Dessutom rekryterades tolv friska frivilliga för att samla in data för syftet att utvärdera retrospektiva statistiska metoder för förbättring av data. Totalt genomgick varje frivillig tre referensmätningar och tre mätningar med huvudrörelser som utfördes enligt ett rörelseparadigm. 'Order Statistic Filtering' (OSF) och 'Jackknife Correlation' (JKC) utvärderades med avseende på artefakt-eliminering och om reliabiliteten i de resulterande metabolit-

koncentrationerna ökades. Slutligen utvärderades även den nya MEGA-editerade MRSI-sekvensen med fantommätningar.

För narkolepsipatienterna observerades en association på trendnivå mellan de frontala MRS koncentrationerna och ökad deaktivering i 'default mode network' under minnesuppgiften. Hos IBS-patienterna med svåra ångestsymtom observerades en signifikant högre frontal GABA+ koncentration. Slutligen, för ET-patienterna observerades en positiv korrelation mellan kvoten GABA+ och Glx i lillhjärnan och graden av tremor hos patienterna.

Eftersom huvudrörelser under MRS-mätningen genererade signalförlust i spektrumen resulterade detta i reducerade metabolitkoncentrationer. Både OSF och JKC gav en förbättring av de signalintensiva metaboliterna i spektrumet när artefakter fanns i spektrumen, samtidigt som båda metoderna presterade likvärdigt som konventionell analysmetod när referensmätningarna analyserades. Slutligen, med den MEGA-editerade MRSI-sekvensen utvecklad för en konventionell klinisk MR-kamera visade fantommätningarna att GABA var detekterbart med en 1:45 min lång mätning där varje enskild voxel rymde 1 mL.

Flera utmaningar såsom tidsmässiga begränsningar, stora voxlar, och design av mätprotokoll, påträffades när MEGA-editerad SVS användes i de kliniska applikationerna. Dessutom, artefakter i spektrumen som uppkommit från exempelvis rörelse påverkade de resulterade spektrumen negativt, vilket både var tydligt i de kliniska mätningarna och i mätningarna utförda på de friska frivilliga. När artefakter fanns i spektrumen förbättrade både OSF och JKC de signalintensiva metabolitkoncentrationerna i spektrumet, och JKC var att föredra över OSF. Dessutom visades det att JKC potentiellt även kan användas som en teknik för kvalitetskontroll av en mätning eller som en indikation på om voxeln flyttats via rörelse under mätningen.

Att använda MRSI-sekvensen medför många tekniska utmaningar, men även om de tekniska utmaningarna är många, skulle en optimal metodologi för GABA-kvantifiering vara extremt värdefull. Speciellt i kliniska forsknings-applikationer där sjukdomar såsom narkolepsi, IBS, eller ET undersöks, där minimala voxlar och kort mättid är väldigt önskvärt.

LIST OF PAPERS

I. Evidence for Cognitive Resource Imbalance in Adolescents with Narcolepsy

S. Witt, N.M Drissi*, **S. Tapper***, A. Wretman, A. Szakács, T. Hallböök, A.M. Landtblom, T. Karlsson, P. Lundberg, M. Engström
Brain Imaging and Behavior. 2018; 12(2): 411-424.

II. Increased Inhibitory Neurotransmitter Concentration in Medial Prefrontal Cortex is Associated with Anxiety in Irritable Bowel Syndrome

A. Icenhour, **S. Tapper**, O. Bednarska, S. Witt, A. Tisell, P. Lundberg, S. Elsenbruch, S. Walter
In manuscript.

III. Essential Tremor: Cerebellar GABA+/Glx Ratio is Correlated with Tremor Severity

S. Tapper*, N. Göransson*, P. Lundberg, A. Tisell, P. Zsigmond
In manuscript.

IV. How Does Motion Affect GABA Measurements? Order Statistic Filtering Compared to Conventional Analysis of MEGA-PRESS MRS

S. Tapper, A. Tisell, P. Lundberg
PLoS One. 2017; 12(5): e0177795. doi: 10.1371/journal.pone.0177795

V. Retrospective Artifact Elimination in MEGA-PRESS using a Correlation Approach

S. Tapper, A. Tisell, G. Helms, P. Lundberg
Magnetic Resonance in Medicine, 2018;00:1-15.

VI. nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges

S. Tapper, A. Tisell, P. Lundberg
In manuscript.

* Authors contributed equally to the manuscript.

Other Contributions

1. **Reduced excitatory neurotransmitter levels in anterior insulae are associated with abdominal pain in irritable bowel syndrome**
O. Bednarska, A. Icenhour, **S. Tapper**, S. Witt, A. Tisell, P. Lundberg, S. Elsenbruch, M. Engström, S. Walter
In Manuscript
Peer reviewed conference abstracts:
1. **The Influence of Bold-fMRI (GRE-EPI) on MEGA-PRESS Measurements of GABA Concentrations**
S. Tapper, A. Tisell, P. Lundberg
Joint Annual Meeting ISMRM-ESMRMB, Milan, Italy, **2014**
2. **Neurotransmitter Concentration in Pregenual ACC in Stool Consistency Patient Subgroups with IBS**
O. Bednarska, **S. Tapper**, P. Lundberg, M. Lowén, S. Walter
UEG Week, Vienna, Austria, **2014**
3. **Subject Movements in MRS: Evaluating the Reliability in Concentrations Determined at 3 T**
S. Tapper, A. Tisell, P. Lundberg
ESMRMB, Edinburgh, United Kingdom, **2015**
4. **Increased Inhibitory Neurotransmission within Anterior Cingulate Cortex is Related to Comorbid Anxiety in Irritable Bowel Syndrome**
A. Icenhour, O. Bednarska, **S. Tapper**, P. Lundberg, S. Witt, S. Elsenbruch, S. Walter
UEG Week, Vienna, Austria, **2016**
5. **Motion Compensated MRS: Order Statistics Applied on MEGA-PRESS Datasets**
S. Tapper, A. Tisell, P. Lundberg
ISMRM Workshop on MR Spectroscopy, Lake Constance, Germany, **2016**
6. **Order Statistic Filtering Performed on MEGA-PRESS Data Influenced by Subject Head Motion**
S. Tapper, A. Tisell, P. Lundberg
ESMRMB, Vienna, Austria, **2016**
7. **Initial Experience with a 3D MEGA-semi-LASER MRS Sequence**
S. Tapper, A. Tisell, P. Lundberg
ESMRMB, Barcelona, Spain, **2017**
8. **Increased Rostral Anterior Cingulate Cortex GABA+ Concentrations in IBS Patients with Anxiety**
S. Tapper, A. Icenhour, O. Bednarska, A. Tisell, S. Walter, P. Lundberg
ISMRM, Paris, France, **2018**
9. **Artifact Detection Using Correlation Analyses Applied to MEGA-PRESS Data Containing Subject Head Movements**
S. Tapper, A. Tisell, G. Helms, P. Lundberg
ISMRM, Paris, France, **2018**

ABBREVIATIONS

ACC	Anterior Cingulate Cortex
ATP	Adenosine TriPhosphate
BOLD	Blood-Oxygen Level Dependent
Cho	Choline
CNS	Central Nervous System
Cr	Creatine
CSDE	Chemical Shift Displacement Error
CSF	CerebroSpinal Fluid
CSI	Chemical Shift Imaging
DMN	Default Mode Network
EEG	ElectroEncephaloGram
ET	Essential Tremor
ETRS	Essential Tremor Rating Scale
fMRI	functional Magnetic Resonance Imaging
GABA	Gamma-Amino Butyric Acid
GABA+	GABA plus co-edited signals
Gln	Glutamine
Glu	Glutamate
Glx	Glutamine and glutamate
GM	Gray Matter
HADS	Hospital Anxiety and Depression Scale
IBS	Irritable Bowel Syndrome
LASER	Localization by Adiabatic SElective Refocusing
MEGA-PRESS	MEscher-GARwood Point RESolved Spectroscopy
mPFC	medial PreFrontal Cortex
MRI	Magnetic Resonance Imaging
MRS	Magnetic Resonance Spectroscopy
MRSI	Magnetic Resonance Spectroscopy Imaging
NAA	N-AcetylAspartate
NAAG	N-AcetylAspartylGlutamate
NMR	Nuclear Magnetic Resonance
PC	PhosphoCholine
PCr	PhosphoCreatine
PFC	PreFrontal Cortex
ppm	Parts per million

PRESS	Point RESolved Spectroscopy
RF	Radio Frequency
sLASER	semi-LASER
tCho	Total Choline compounds
tCr	Total Creatine compounds
tNA	Total N-Acetyl compounds
WM	White Matter

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1. INTRODUCTION

Although the human brain has been studied for many years, the mechanisms behind the complex functions of both the healthy and diseased brain are still not completely understood. Magnetic Resonance Spectroscopy (MRS) is a completely non-invasive method that can be used to study various metabolites within the brain. For example, neurotransmitter concentrations of GABA and glutamate can be studied using special experimental designs. GABA, or γ -aminobutyric acid, is the main inhibitory neurotransmitter in the mature human central nervous system and is synthesized from glutamate, the main excitatory neurotransmitter. Furthermore, many studies have shown associations between regionally altered GABA or glutamate concentrations and neurological disorders such as Parkinson's disease, epilepsy, and schizophrenia. In addition, associations between altered neurotransmitter concentrations and psychological symptoms such as anxiety and depression have also been reported.

Although MRS has been around since the mid-1970s, it has not yet had a major clinical breakthrough, which is probably due to the many challenges that are associated with *in vivo* MRS. Long measurement times and large voxels are challenges that are particularly prominent when spectral editing procedures are used in order to quantify low-intense metabolites such as GABA. In addition, subject movements can be expected during a longer MRS measurement, which will induce artifacts in the resulting spectrum. Therefore, there is much room for methodological improvements in terms of increasing the reliability of the collected data using statistical approaches in order to filter out artifact-influenced spectra.

Magnetic Resonance Spectroscopy Imaging (MRSI) approaches could be performed in order to cover a large region of the brain within one measurement. In clinical research MRS applications, there is a great desire for an MRS measurement with a high spatial resolution, a short measurement time, and at the same time with large brain coverage. However, in terms of these factors, there are limits to what is technically possible while ensuring the MRS measurement remains reliable. Although there are many technical challenges associated with GABA quantification, it is worth pursuing since a fast and reliable MRSI GABA measurement would be very valuable in order to further elucidate the mechanisms behind certain neurological disorders. This MRSI GABA measurement would be especially well-suited for the clinical research setting, where scanner time is precious and the targeted brain regions often are very small.

1.1 Functions and Diseases of the Central Nervous System

The central nervous system (CNS) is a very complex organ that is primarily composed of neurons. A neuron is an electrically excitable cell that receives, processes, and transmits information through both electrical and chemical signals. Moreover, a typical neuron consists of a cell body with dendrites and an axon, and neurons communicate with each other via connections called synapses. The CNS consists of gray and white matter (GM and WM), where GM mainly consists of neuronal cell bodies and unmyelinated fibers, while WM mainly consists of myelinated axons (Figure 1.1 A). Both GM and WM also includes glial cells, or supporting cells, where for example the star-shaped astrocytes are involved in both clearance of metabolites as well as transport of substances to the neurons from the capillaries of the brain [1].

1.1.1 Neurotransmission: GABA and Glutamate

The CNS circuitry is driven by the process of neurotransmission, which is a process where neurotransmitters are released by the axon terminal of a neuron (presynaptic), and then bind to receptors on the dendrites of another neuron (postsynaptic). These neurotransmitters are released as a response to a threshold action potential and bind to the receptors, which in turn affect the postsynaptic neuron in either an inhibitory or excitatory way. This process depends directly on the availability and release of neurotransmitters, which then bind to the receptor, and the subsequent removal of the neurotransmitter. In addition, a postsynaptic neuron may receive inputs from several neurons, and therefore, it is the net effect that decides whether the response is inhibitory or excitatory, where an excitatory net effect means an increased probability of a neuron to fire.

γ -aminobutyric acid or GABA, is the main inhibitory neurotransmitter in the human CNS [2, 3], and Figure 1.1 B shows an overview of the GABA signaling system in a GABAergic neuron. In contrast to GABA, glutamate is the main excitatory neurotransmitter [4], and both play important roles in ensuing healthy and balanced neurotransmission. Furthermore, since neither of GABA nor glutamate can be synthesized from glucose, both are instead synthesized through the glutamate/GABA-glutamine cycle, where GABA is synthesized from glutamate that previously was synthesized using glutamine as a precursor [5].

Figure 1.1. Illustration of the cellular composition of neuronal tissue (A) and an overview of the GABA signaling system (B). Neuronal tissue mainly consists of neurons, oligodendrocytes, and astrocytes. A neuron typically has several dendrites and one axon, and communication between neurons happens at the synapses. Reprinted with permission from [1] (A) and [6] (B).

1.1.2 The Human Brain

The brain is the main part of the human CNS, and consists of the cerebrum, the brainstem and the cerebellum (Figure 1.2). The cerebrum is the largest part of the human brain, where the cerebral cortex consists of an outer layer of GM that covers an inner core of WM. Furthermore, the cerebrum is divided into four main lobes (frontal, parietal, temporal, and occipital). The first, the frontal lobe has many functions, but is mostly known for forming our personality. The second, the parietal lobe, processes sensory information such as pain, pressure or touch, but is also involved in speech. Thirdly, there are two temporal lobes, each located in close proximity to the ear and thus responsible for interpretation of sound, but also responsible for memory. Finally, the occipital lobe is mainly responsible for visual processing. In addition, also within the cerebrum is the ventricular system where the cerebrospinal fluid (CSF) is produced and circulated.

Underneath the cerebrum, the brainstem connects the brain to the spinal cord and thus has the important function of maintaining communication between the brain and the rest of the body. For example, the brainstem controls crucial cardiac and respiratory functions. Between the cerebrum and the brainstem, the thalamus, amygdala, hippocampus, and hypothalamus are located. The latter connects the nervous system to the endocrine system via the pituitary gland.

Furthermore, the cerebellum is located beneath the cerebrum, and plays a major role in motor control and coordination.

Figure 1.2. Illustration of the brain. The four main lobes (frontal, parietal, temporal, and occipital) of cerebrum, the brainstem, and the cerebellum are shown. Image from Wikimedia commons.

1.1.2.1 Prefrontal- and Anterior Cingulate Cortex

The prefrontal cortex (PFC) and anterior cingulate cortex (ACC) together form the anterior parts of the frontal lobe (Figure 1.3 A). Although the PFC and ACC have long been thought to play a crucial role in the processing of emotions, and thus, also in the manifestation of our personality, the exact functional contribution still remains uncertain. Several studies have shown that the PFC is highly interconnected to other regions of the brain involved with functions such as attention, cognition, and mental state. Another widely accepted theory is that the PFC serves as a storage for short-term memory, also known as the working memory. Furthermore, the medial PFC (mPFC) in particular has been shown to be a key node in emotion regulation and top-down corticolimbic inhibitory control. Meanwhile, the ACC has shown involvement in pain perception and in the mediation of the emotional response to pain, and has also been linked to task effort and attention [7, 8]. However, there is greater uncertainty about the exact function of the PFC and ACC since some of the functions described above may in turn map into distinct sub-regions of both the ACC and PFC. Recently, many studies have investigated these regions or sub-regions in patients with neurological disorders such as schizophrenia and bipolar disorder, but their function and structure have also been investigated and associated with *e.g.* sleep, memory, anxiety and depression [7, 8].

1.1.2.2 Thalamus

The thalamus is a paired GM structure joined at the midline of the brain, which consists of many different nuclei and is located according to Figure 1.3 B [9]. Since the thalamus has nerve fibers projecting out in all directions to the rest of the brain, it is generally believed to act as a hub that relays information between

different subcortical areas and the cerebral cortex. For example, information from the sensory organs (except the olfactory system), is relayed via a specific nucleus in the thalamus to the specific responsible region within the cerebral cortex. Other parts of the thalamus are believed to play important roles in sleep, awareness, memory, and language. In addition, some thalamic nuclei have also been suggested to act as a subcortical movement center due to the nature of the interconnection between the cerebellum, thalamus, and motor cortex [9].

1.1.2.3 Cerebellum

The cerebellum plays an important role in motor control in humans by assisting in the coordination, precision, and timing of movements (Figure 1.3 C) [10]. The cerebellum receives input from the sensory systems of the spinal cord and from the cerebral cortex, and processes these signal inputs to fine-tune motor activity. Therefore, cerebellar damage usually produces disturbed fine movements, balance, or posture. Other potential functions such as cognitive attention, pain avoidance, or language are less established. In contrast to the cerebral cortex, the cerebellum consists of a continuous tightly folded layer of GM, constructed of several types of neurons such as Purkinje cells (GABAergic) and granule cells (glutamatergic) [11, 12]. Under this folded layer, four deep nuclei (GM) are embedded within the WM interior of the cerebellum, and these receive inputs from mossy fibers and climbing fibers as well as from the Purkinje cells. Furthermore, the dentate nucleus is the largest of the four deep nuclei that send output (glutamatergic) from the cerebellum to the rest of the brain [10, 11].

Figure 1.3. Illustration of the PFC (red) and ACC (blue) (A), thalamus (B), and cerebellum (C). This figure was constructed using images from Wikimedia commons.

1.1.3 Narcolepsy

Narcolepsy is a chronic sleep disorder characterized by excessive sleepiness, uncontrollable sleep attacks, sleep paralysis, and experienced hallucinations during onset of sleep or when going from sleep to a wakeful state. Cataplexy is another common symptom that affects around 70% of patients with narcolepsy, which is manifested by a sudden loss of muscle control induced by emotional stimuli such as laughing or crying [13]. Furthermore, the exact cause of narcolepsy is unknown, but some parts of the disease involves a loss of orexin-releasing neurons in the hypothalamus [14]. In addition, around 10% of cases are genetic, and other factors may be environmental ones (toxins), infection, brain injury, or autoimmunity. Finally, a link between the H1N1 Pandemrix vaccination in Sweden and Finland, and an increased risk of developing narcolepsy in adolescents was confirmed by the Swedish Medical Products Agency in 2013.

1.1.3.1 Working Memory in Narcolepsy

Patients with narcolepsy frequently report subjective complaints about working memory, but studies have failed to find empiric evidence for a genuine memory deficit in narcolepsy [15-17]. Instead, most of the evidence points toward deficits in sustained attention, and this pattern of cognitive dysfunction in narcolepsy is thought to be consistent with an imbalance of cognitive resources related to changes in the orexin-system [18-20]. However, more research is needed to further elucidate the mechanisms behind narcolepsy.

1.1.4 Irritable Bowel Syndrome

Irritable Bowel Syndrome, or IBS, is a common disorder characterized by abdominal pain and disturbed bowel habits without any evidence of underlying damage. Diagnosis of IBS is based on these symptoms in the absence of factors such as inflammatory bowel disease, blood in the stool, weight loss, or celiac disease. Furthermore, IBS is estimated to affect around 7-21% of the general population, and is more common in women. There is no cure for IBS and treatment is currently carried out to improve the symptoms *e.g.* by dietary changes, medication, and counseling [21-23].

1.1.4.1 Comorbid Psychological Symptoms

A large proportion of IBS patients experience comorbid psychological symptoms [24-26], and studies have observed altered mPFC function in IBS with comorbid anxiety and depression [27, 28]. Furthermore, many studies have previously reported altered neurotransmitter concentrations in patients with psychological symptoms. Also a direct link between anxiety and increased mPFC GABA concentration has been observed in healthy volunteers [29, 30], which may suggest that a dysregulation of prefrontal inhibitory control may be involved in

aberrant emotion regulation and psychological complaints [31]. This hypothesis may also be valid for IBS.

1.1.4.2 Brain-Gut Axis

The exact causes of IBS are currently not clear, and one potential explanation is a disturbance in the brain-gut communication (Figure 1.4) [32]. A disturbance of this bidirectional communication between the brain and the gastrointestinal system has been increasingly acknowledged in various neuropsychiatric disturbances, and thus, IBS with comorbid psychological symptoms is a fitting example [33]. Finally, the current evidence points toward the role of the brain in IBS, which needs to be further investigated.

Figure 1.4. Simplified illustration of the brain-gut communication. Factors such as stress, anxiety, and depression may be caused by or cause the gastrointestinal issues frequently reported in IBS. (Parts of the image created using Mind the Graph)

1.1.5 Essential Tremor

Essential tremor (ET) is a common progressive movement disorder, which has a prevalence of 4.6% (age ≥ 65 years) [34]. In contrast to Parkinson's disease, the tremor is characterized by an action tremor, which means that the tremor intensifies when the patient tries to perform voluntary movements such as eating or writing. The onset of tremor is most common in the upper limbs, and in the majority of cases, the tremor spreads to the rest of the body during the progression of the disease [35]. In addition, cognitive and psychiatric symptoms can also appear, which thus in total causes ET to be a very heterogenic disease [35, 36].

The underlying mechanisms behind ET are still not fully understood; however, several studies support the involvement of the cerebellum and thalamus. For example, postmortem studies have shown structural changes in the cerebellum, including a reduced number of Purkinje cells, which propagate input to the dentate nucleus through GABA [37]. In addition, a decreased number of GABA receptors in the dentate nucleus has also been observed [38].

1.1.5.1 Deep Brain Stimulation Intervention

In severe cases of ET, Deep Brain Stimulation (DBS) surgery can be performed in order to alleviate the tremor. The electrodes are usually placed either in the ventral intermediate nucleus of the thalamus (VIM) or in the zona incerta. Finally, since the mechanisms behind DBS are still not completely understood, further studies are needed, for instance to elucidate the role of DBS in neurotransmission.

1.2 A Brief Introduction to Nuclear Magnetic Resonance

The phenomenon of Nuclear Magnetic Resonance (NMR) is only exhibited by nuclei with an odd number of either protons or neutrons. These nuclei possess a property known as spin angular momentum (either up or down), which can be visualized as a spinning motion about their own axis. When many nuclei with this spin property (such as protons, ^1H) are placed in a static magnetic field (B_0 , usually applied in the z-direction), the spins are divided into two energy levels. There are slightly fewer spins in the lowest energy level since more nuclei are aligned with the magnetic field than against it, and thus the net magnetization is aligned with the static magnetic field [39].

In 1946, Bloch and Purcell showed that a sample of spins will interact with an oscillating magnetic field of a particular angular frequency ω (in the radio frequency range), if ω exactly matches the energy difference between the spin up and down levels [40, 41]. This angular frequency at which the spins interact with the magnetic field is termed the Larmor frequency or resonance frequency (ω_0). The relationship between ω_0 and B_0 is described by the Larmor equation (Eq. 1.1), where γ is the gyromagnetic ratio (42.6 MHz/T, ^1H), and $\omega_0 = 2\pi\nu_0$. Nuclear isotopes have unique gyromagnetic ratios, and therefore, different nuclei resonate at different frequencies when exposed to the same magnetic field strength.

$$\omega_0 = -\gamma \cdot B_0 \quad \text{Eq. 1.1}$$

1.2.1 Excitation, Signal, and Relaxation of Resonances

At equilibrium, the net magnetization M is aligned with B_0 in the z-direction, and termed $M_z = M_0$. In addition, since the spins do not have any preferred direction in the x-y plane, this magnetization is termed $M_{xy} = 0$. To observe the NMR phenomenon, M needs to be perturbed away from the z-direction. Therefore, a transverse magnetic field B_1 is applied in the x-y plane with a radio frequency (RF) exactly tuned to ν_0 , and thus fulfilling Eq. 1.1. Using this B_1 field, M can for example be rotated down into the x-y plane using a 90° RF pulse, which means that B_1 is turned off when M intersects the x-y plane. After this 90° RF pulse, a net magnetization exists in the x-y plane (transverse magnetization) and due to the inherent property of spin, this transverse magnetization rotates with the frequency ν_0 . This transverse magnetization can be detected using a receiver coil exactly tuned to ν_0 to detect the RF voltage induced by M_{xy} .

The process of M returning to the equilibrium state is called relaxation. First, the return of the z-magnetization to M_0 is described by the longitudinal, or spin-lattice relaxation time, which is characterized by the time constant T_1 . Second, the dephasing of spins in the x-y plane is described by the transversal, or spin-spin relaxation time, which in turn is characterized by the time constant

T_2 . In addition, there are also external factors such as magnetic field inhomogeneities caused by e.g. eddy currents, metal, or gradients. These effects accelerate the dephasing of spins in the x-y plane, and can be described by the time constant T_2^* .

The dephasing effect can be reversed by applying a 180° RF pulse, and this refocusing pulse will cause the spins to start to gain phase coherence. When all spins are coherent again, a spin echo is generated before the dephasing starts over. The combined effect of the relaxation and the induced signal through rotating magnetization in the x-y plane result in a signal that is called the 'free induction decay' (FID), which is described by the frequency ν_0 and the exponentially decaying time constant T_2^* .

1.3 ^1H Magnetic Resonance Spectroscopy

Proton Magnetic Resonance Spectroscopy (^1H MRS) is a non-invasive tool used in both clinical and research applications. The FID measured by the scanner can be described as a combination of decaying exponential functions corresponding to the resonance signals. To aid in the analysis process of these signals, a spectrum is computed by applying the fast Fourier transform to the FID. This resulting spectrum contains information about the molecules within the examined sample. Both the chemical shift and the spin-spin coupling are properties directly related to the structure of the molecule, which create the resulting spectral appearance of that particular molecule.

1.3.1 Chemical Shift

The signal from the same nuclei on different molecules, or positions within the molecule does not resonate at the same frequency. When placed in an external magnetic field, the nuclei experience different magnetic fields depending on the surrounding electrons. These electrons shield the nuclei by producing a small opposite magnetic field as a response to B_0 . The resulting shift in resonance frequency is termed the chemical shift, and is defined as the difference in resonance frequency from a chemical shift reference resonance standard compound (such as tetramethyl-silane or equivalent water soluble alternatives assigned to $\nu_0 = 0.00$ ppm), measured in parts per million (ppm) relative to the reference resonance (Eq. 1.2). Finally, a lower chemical shift

$$\delta = \frac{\nu - \nu_0}{\nu_0} \cdot 10^6 [\text{ppm}], \quad \text{Eq. 1.2}$$

means a higher shielding, and thus a higher electron density, where ν_0 is the resonance frequency of the shift reference standard.

1.3.2 Spin-Spin Coupling

Spin-spin coupling, J-coupling, or scalar coupling, is a phenomenon where a resonance can split into several separate lines in the spectrum. This splitting is caused by spin-spin interactions that act through the bonds within the molecule. Furthermore, sometimes this splitting can cause very complex patterns in the spectrum since several multiplets may overlap, especially at lower magnetic field strengths. However, the properties of the splitting patterns such as the number of peaks in a multiplet, spacing, and their relative intensities, follow certain rules [39]. For example, the separation between two different lines can be described by the coupling constant (J , measured in Hz), which is independent of the magnetic field strength.

1.3.3 The Single-Voxel MRS Experiment

Single-voxel spectroscopy (SVS) has been used in a variety of clinical research applications on the brain such as dementia, aging, infections, psychiatric disorders, and cancer. Depending on what the aim is of the study, and thus what metabolites are targeted, certain parameters such as repetition time (TR) and echo time (TE) need to be chosen properly. Figure 1.5 shows an example of a resulting spectrum from an MRS measurement performed with the voxel placed in the cerebellum.

Figure 1.5. Example of an MRS measurement. The measurement (about 10 min) was performed with the voxel placed in the cerebellum using a $TR = 2$ s, and $TE = 68$ ms. The resulting spectrum is assigned according to 1, creatine ($^{-2}CH_2$); 2, total glutamine and glutamate (Glx; ^{-2}CH); 3, choline ($-N[CH_3]_3$); 4, creatine ($-N[CH_3]$); 5, total N-Acetyl compounds (tNA; $^{-3}CH_2$); 6, Glx ($^{-4}CH_2$); 7, tNA ($^{-2}CH_3$).

1.3.3.1 Shimming

Before making the MRS measurement, it is important that the magnetic field within the voxel is homogeneous. Therefore, a shimming procedure is performed using shim coils in order to correct for any field inhomogeneity. Poor shimming of the voxel can be observed in the resulting spectrum by a widening of the resonances, which complicates the quantification of the metabolite concentrations.

1.3.3.2 Water Suppression

Since proton spectroscopy targets the hydrogen signal, and the water concentration is about 10,000 times larger than the other metabolites within the brain, water suppression is usually used. Figure 1.6 illustrates the effect of using water suppression, and from this figure it is possible to see that the unsuppressed water signal (at 4.7 ppm) is contaminating the metabolite signals in the 1-4 ppm range. However, a separate shorter water reference is usually acquired in order to obtain a reference of water within the tissue in the voxel, and also, it may be used for eddy current correction in the post-processing step.

Figure 1.6. The effect of using water suppression. (A) The water signal resulting from a water reference measurement, and zooming in on the metabolite-window were the interesting metabolite are (1-4 ppm). (B) The spectrum when water suppression was used, here using weak water suppression (MOIST).

1.3.3.3 Localization of the Voxel: PRESS

Several voxel localization techniques such as PRESS were developed in the mid-1980s. The PRESS (Point-Resolved Spectroscopy) pulse sequence [39, 42] consists of a 90° RF excitation pulse followed by two 180° refocusing pulses that are carried out in combination with magnetic field gradients. These gradient pulses are orthogonal, and therefore, only the intersections of the resulting three slices are refocused; meanwhile, all other signals are being dephased. The minimum echo time (TE) available is determined by the length of the RF and gradient pulses, which on conventional clinical MR systems is around 30 ms.

1.3.3.4 Chemical Shift Displacement Error

The chemical shift displacement error (CSDE) is a large problem particularly when using a pulse sequence from the PRESS family at higher field strengths (3 T and above) and with a limited available B_1 (Figure 1.7). As a consequence of this error, the voxel from which for example the N-acetylaspartate signal (NAA, 2.0 ppm) originates does not spatially match the measured voxel for creatine (Cr, 3.0 ppm). This phenomenon is manifested since an RF pulse with a given frequency range will excite slightly different regions for spins chemically shifted with a frequency offset Δf [39].

Figure 1.7. Illustration of the chemical shift displacement error (CSDE). A RF-pulse excite slightly different regions for spins chemically shifted with a frequency offset Δf . The N-acetyl aspartate (NAA, 2.0 ppm), creatine (Cr, 3.0 ppm), and choline (Cho, 3.2 ppm) are large signals in the spectrum, and due to the CSDE, the corresponding voxels may not spatially match.

1.3.3.5 Adiabatic Selection

A possible solution to the CSDE is to replace the PRESS localization with a LASER (Localized by Adiabatic Selective Refocusing) or a semi-LASER (sLASER) pulse sequence, which utilizes adiabatic pulses [43, 44]. These adiabatic pulses are a class of amplitude- and frequency-modulated RF pulses. In contrast to amplitude-modulated rectangular RF pulses where all spins are affected simultaneously, the adiabatic pulses manipulate spins with different resonance frequencies at different times. Therefore, adiabatic pulses have the advantage of being relatively insensitive to both B_1 inhomogeneity and frequency offset effects. In addition, the adiabatic pulses also have the advantage of having a high tolerance to field inhomogeneity, and minimize the associated heating of the sample during the measurement.

Adiabatic excitation or refocusing occurs only under certain limited conditions and produces a complex motion of the net magnetization M , since B_1 is applied off-resonance (thus not exactly at the resonance frequency); therefore, instead of precessing around B_1 , M precesses in a cone around an effective field (B_e) that is angled to both B_1 and B_0 . M will gradually follow this

effective field if the adiabatic condition is fulfilled (Eq. 1.3, where $\Delta v(t) = v(t) - v_c$, and v_c is the RF carrier frequency [39]), which means that B_1 should be sufficiently strong and applied slowly to still fulfill the condition below,

$$|B_e(t)| = \sqrt{B_1^2(t) + \Delta v^2(t)} \gg \left| \frac{d\alpha(t)}{dt} \right|, \quad \alpha(t) = \tan^{-1} \frac{\Delta v(t)}{B_1(t)}. \quad \text{Eq. 1.3}$$

1.3.3.6 Phase Cycling

Phase cycling can be performed in order to improve the localization, and is usually performed using between two to 16 phase cycle steps. Despite the improvement in the voxel localization, it is time-consuming and can induce further artifacts when used in combination with editing techniques [45].

1.4 Magnetic Resonance Spectroscopy Imaging

Magnetic Resonance Spectroscopy Imaging (MRSI) or chemical shift imaging refers to multi-voxel techniques that uses phase encoding for spatial localization [46-48]. Thus, instead of using a single voxel in the measurement, for example a 2D or 3D grid of voxels can be utilized. MRSI allows wide total brain coverage and a high spatial resolution. In clinical applications, these are two very important advantages, especially since the regions of interest in the brain are often small. In addition, in spectroscopy studies, several regions are also often targeted within the same protocol, and when using SVS, this would require separate measurements and thus be very time-consuming. There are also several disadvantages of MRSI such as shimming issues, lower spectral quality of individual voxels, and contamination of signals from adjacent voxels. However, using conventional MRSI, the major disadvantage is instead the long imaging time, which is due to the readout where the phase encoding gradients need to step through all voxels in a nested fashion. Fortunately, there are several approaches to reduce the acquisition time, among which spiral readout is a strategy that was first used in the mid-1980s.

1.4.1 Spiral Readout

Spiral is a fast technique that can be used for readout of MRSI data. Spiral is a variation of the EPSI technique (Echo-Planar Spectroscopic Imaging) [49]. In the EPSI technique, an oscillating readout gradient collects a single line in k-space at different time points, which allows for simultaneous acquisition of spatial and spectral information. At the same time, phase encoding occurs on the other axes, which allows for 2D or 3D localization. In contrast to EPSI where lines are read, two oscillating gradients are instead applied along two axes in order to create a spiral trajectory starting from the center of k-space (k_0), which then curves outward in k-space. Moreover, not only is the spiral readout a very fast approach, since k_0 is oversampled, spiral is also rather insensitive to

movements. However, phase errors can accumulate during the readout, and high demands are put on the gradients in order to perform correct a readout [50, 51].

Spiral readout is usually performed by using a number of angular and temporal interleaves, which are illustrated by Figure 1.8. The angular interleaves read k-space in a predefined number of directions, while the temporal interleaves are performed with a time delay in order to increase the temporal resolution of the resulting FID. Choosing the specific spiral parameters such as number of interleaves, readout duration, and sample points, involves a trade-off between acquisition time, spatial resolution, and readout duration.

Figure 1.8. Schematic illustration of the spiral readout. The resulting FID is shown when using two temporal interleaves are used in the spiral readout in combination with four angular interleaves (i.e. four ‘spiral arms’, α_{1-4}). Every other samples (blue) in the FID originate from temporal interleave 1, which was collected without any time delay. The green samples in the FID originate from temporal interleave 2, which were collected with a time delay Δt , which corresponded to half the time between two following samples. (Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript).

1.5 Metabolites

This section describes the spectral appearance and some properties of the different metabolites targeted in this thesis.

1.5.1 GABA

As previously described, GABA is the main inhibitory neurotransmitter in the CNS, which gives three coupled resonances in the spectrum at 1.9, 2.3, and 3.0 ppm (Figure 1.9). In the brain, the concentration of GABA is about 1 mM, and it has been reported to be higher in GM and to decline with age [3, 52]. Because of the low signal intensity of GABA compared to other signals in the spectrum, and due to overlap of other signals, spectral editing techniques are commonly used in order to quantify GABA (described further below).

Figure 1.9. GABA. Molecular structure of GABA together with a high resolution spectrum simulated at 4.0 T, where the gamma-protons appear at 3.0 ppm and the beta-protons at 1.9 ppm.. Reprinted with permission from [53].

1.5.2 Glutamate and Glutamine (Glx)

As previously described, glutamate (Glu) is an excitatory neurotransmitter that is synthesized from glutamine (Gln). Both Glu and Gln are amino acids, and Gln is synthesized from Glu using Adenosine Triphosphate (ATP). The typical concentrations of both Glu and Gln in the brain are relatively high (<12 mM for Glu, and 4-6 mM for Gln). Generally, it has been observed that Glu is higher in neurons while Gln is higher in astrocytes [3]. An increased Glu level has been interpreted as indicating increased metabolite activity. However, as is apparent in Figure 1.10, it is challenging to separate Glu and Gln using MRS, and often these two metabolites are quantified in a combined measurement termed Glx.

Figure 1.10. Glutamate (Glu) and glutamine (Gln). Molecular structure of Glu and Gln together with high resolution spectra simulated at 4.0 T. Reprinted with permission from [53].

1.5.3 Creatine

The total creatine (tCr) resonance at 3.02 ppm (Figure 1.11) originates from both creatine (Cr) and phosphocreatine (PCr), which are two compounds that are in a fast near-equilibrium exchange on the NMR time scale. Furthermore, PCr has significantly shorter T_2 than Cr, and therefore, these two compounds will contribute differently to the tCr resonance in the spectrum, depending on the chosen TE. Cr is both synthesized in the brain and uptaken through the blood via the diet, and has an important role in energy metabolism. For example, PCr has an ATP buffering role in the brain, and the PCr level has been shown to decrease in relation to functional activation [3]. In clinical MRS studies, creatine is often used as a reference compound, which for the reasons above can be problematic.

Figure 1.11. Creatine. Molecular structure of phosphocreatine and creatine together with high resolution spectra simulated at 4.0 T. Reprinted with permission from [53].

1.5.4 Choline

The choline resonance at 3.2 ppm (tCho) arises from glycerophosphocholine (GPC), phosphocholine (PC), and free choline (Figure 1.12). Choline can be seen as a membrane marker, where the tCho resonance can reflect non-steady-state alterations in membrane turnover by either increased synthesis or breakdown, or changes in cell density [3].

Figure 1.12. Choline. Molecular structure of choline and glycerophosphocholine (aka glycerophosphorylcholine) together with high resolution spectra simulated at 4.0 T. Reprinted with permission from [53].

1.5.5 N-Acetylaspartate and N-Acetylaspartylglutamate (tNA)

The N-acetyl resonance at 2.0 ppm (tNA) is mainly contribution from N-acetylaspartate (NAA) with a smaller contribution of N-acetylaspartylglutamate (NAAG) (Figure 1.13). The relationship between NAA and NAAG is similar to the Glu and GABA relationship; however, NAA does not appear to act as a neurotransmitter. In addition, NAA has been considered as a neuronal marker, either as a density marker or as a marker for neuronal integrity [3].

Figure 1.13. NA-compounds. Molecular structure of N-Acetylaspartate (NAA) and N-Acetylaspartylglutamate (NAAG) together with high resolution spectra simulated at 4.0 T. Reprinted with permission from [53].

1.6 Spectral Editing for GABA Detection

Since the spin-spin coupling effects within the GABA molecule result in a complex pattern of low-intense signals, the GABA quantification is usually difficult. In addition, the GABA quantification is further complicated due to overlapping of the more signal-intense creatine resonance at 3.0 ppm. Therefore, an editing technique is usually used in order to detect GABA, and Mescher-Garwood (MEGA) editing is the most commonly used technique for this purpose [45, 54-56]. In MEGA editing, the known spin-spin couplings within the GABA molecule are used in order to separate GABA from the other signals in the spectrum. This editing procedure is performed using a frequency-selective editing pulse applied at two alternating frequencies. For the purpose of GABA detection, the editing pulse is commonly applied alternately at 1.90 ppm (ON) and 7.46 (OFF), thus symmetrically placed around the water signal at 4.68 ppm. During ON editing, this pulse affects the spins coupled to spins at 1.90 ppm, meanwhile, during OFF editing, these spins are allowed to evolve freely. Finally, the difference spectrum is analyzed, which reveals all signals that were affected by this ON editing pulse (Figure 1.14). The TE is usually set to 68 ms ($1/(2J) = 68$ ms) for optimal GABA detection, where the coupling constant $J = 7.3$ Hz between the GABA spins at 1.9 ppm and 3.0 ppm.

In SVS applications, MEGA editing is commonly combined with PRESS localization (MEGA-PRESS), but variations using sLASER also exist [57-60]. Moreover, Mikkelsen *et al.* (2018) showed in a multi-site and multi-vendor study

that the CV of the resulting GABA concentrations was 12% for all datasets, and the mean within-site CV was 10% [59]. Finally, there are also applications where MEGA editing has been combined with MRSI [61].

Figure 1.14. Pulse sequence and illustration of the effect of using MEGA editing from an actual *in vivo* measurement. The editing pulse is alternately applied at 1.90 ppm (ON), which due to spin-spin coupling within the GABA molecule also affects the GABA signal at 3.0 ppm, and at 7.46 ppm (OFF), which do not affect the signals in the 1-4 ppm window. The overlapping creatine signal at 3.0 ppm is eliminated by subtracting the OFF spectrum from the ON spectrum, which in turn reveals the GABA signal at 3.0 ppm. Pulse sequences reprinted with permission from [45].

1.6.1 Limiting Aspects of the MEGA-PRESS Experiment

Due to the low signal intensity of GABA, many signal averages and a large voxel are needed in order to achieve a high enough signal-to-noise-ratio (SNR) in the resulting difference spectrum. Commonly, the measurement is around 10 min long using a voxel of approximately 27 mL. Long acquisition times increase the risk for artifacts in the spectra, originating from *e.g.* subject movements or drifts. In addition, a larger voxel is more difficult to shim, which may lead to increased linewidths in the spectra. Furthermore, when using a J-difference approach such as MEGA editing, there is a large risk for subtraction artifacts in the difference spectra. Therefore, it is important to utilize a proper post-processing routine where phase and frequency correction is performed on all separate spectra before the resulting difference spectrum is computed.

In this thesis, the MEGA-PRESS approach did not consider the effect of the co-edited macromolecular signals. Therefore, the resulting GABA concentrations collected in this thesis were termed GABA+, thus GABA plus the co-edited macromolecular signals. Moreover, there is another approach (Henry *et al.*) by which the OFF pulse is placed instead at 1.5 ppm. Thus, the editing pulses are applied symmetrically around the macromolecular signal at 1.7 ppm [62]. However, this approach increases the TE and reduces the SNR of GABA. Moreover, this approach is also more sensitive to frequency drift.

Although the MEGA-PRESS experiment is optimal for GABA detection here, solely the OFF edited spectra can be used in order to quantify other metabolite concentrations such as tNA, tCr, tCho, and Glx.

1.7 Quantification of MR Spectra Using LCModel

The spectrum contains the sum of all metabolite signals within the voxel. Due to the properties of chemical shifts and coupling constants of the molecules, we know their spectral patterns and can fit functions to the spectrum in order to quantify the metabolites contributing to the signal. There are many software packages available for metabolite quantification. In this thesis, LCModel was used, which is a commercial software package for metabolite quantification that fits the measured spectrum using a linear combination of model spectra (basis set) [63]. Figure 1.15 shows an example output from LCModel.

1.7.1 Quality Parameters

LCModel also estimates some quality parameters for the output. First, each concentration estimate is combined with a %SD parameter, which is a standard deviation measurement expressed as a percentage that is based on the Cramér-Rao lower bound. Multiplication with a factor of two with this parameter gives a rough estimate of the 95% confidence interval. Second, the FWHM (Full width at half-maximum) is a rough estimate of the linewidth in the spectrum. This parameter directly reflects the shim of the voxel, where a lower value

indicates a better magnetic field homogeneity. Third, the S/N reflects the SNR in the spectrum, and in LCModel, S/N is defined as the ratio of the maximum in the spectrum minus baseline to twice the root mean square of the residuals. Furthermore, these three parameters are rough estimates and should only be considered as guidelines.

1.7.2 Water Scaling

As previously described, a separate water reference is often collected within the same protocol as the MRS measurement. The experimental conditions are the same (e.g. voxel position, coil load and temperature) during the acquisition of the water reference and the MRS measurement. Using this reference, water scaling of the concentration estimates can be performed by LCModel. If no water reference is available, the concentration estimates are referenced to Cr, which for the reasons mentioned above (Creatine section) may not be optimal.

Figure 1.15. Example output from LCModel. The resulting red line is fitted to the black measurement data. Above the fit, the residual between the measured signal and the fit is shown. The right panel shows the resulting metabolite concentrations and the quality parameters %SD, FWHM and SNR.

1.8 Statistical Approaches for Improvement of MRS Data

Long acquisition times and certain clinical conditions are circumstances that may lead to signal artifacts induced by movements in the MRS data. Such artifacts influence the resulting spectra negatively, which in turn may lead to unreliable concentration estimates. Furthermore, since many signal averages are collected in each measurement, it is not realistic to individually perform a quality control of each signal average (usually above 300). Therefore, it is important to have some type of algorithm that automatically eliminates potential artifacts present in the data.

1.8.1 Order Statistic Filtering

Order statistic filtering (OSF) or rank-order filtering, is a non-linear filtering approach that has been used in various signal-processing applications since being introduced by Tukey [64]. This rank-ordering approach is robust when the samples are characterized by a non-Gaussian distribution or when the full dataset contains outliers. Furthermore, the MRS signal from one acquisition (m) and sample n can be described by the Eq. 1.4 [65], where S_m is the measured signal, S_m^D is the true signal that is theoretically the same for all acquisitions, δ_m is the noise-term caused by sudden signal artifacts, and ε_m is the noise from the scanner that is considered normally distributed. Signals with high contributions of the δ_m term are considered unreliable since this term is not Gaussian, and therefore, the effects cannot be eliminated using averaging. For example, movement artifacts or sudden scanner instabilities are effects included in this term. Meanwhile, a dataset with a large contribution of ε_m could be seen as reliable, but with a low resulting SNR.

$$S_m[n] = S_m^D[n] + \delta_m[n] + \varepsilon_m[n] \quad \text{Eq. 1.4}$$

Slotboom *et al.* used order statistic filtering on unedited movement-influenced MRS PRESS data, and showed that the resulting spectra became more reliable after filtering [65]. The rank-ordering was performed by ranking all m acquisitions for each individual sample, generating the rank-ordered data r_m . Also, after rank-ordering, the Gaussian noise was removed by estimating ε_m from the end of the signal, where the effect from δ_m was considered minimal (between N_{SNR} and N , Eq. 1.5) [65].

$$O_m[n] = r_m[n] - \frac{1}{N - N_{\text{SNR}}} \sum_{n=N_{\text{SNR}}}^N r_m[n] \quad \text{Eq. 1.5}$$

Figure 1.16 compares standard averaging with OSF, where the original data includes movement artifacts, which are obvious in M_{11} , M_{15} , and M_{29} . Using

OSF each spectrum obtains a resulting increased SNR and the artifacts are shifted out to the beginning and end of the spectral sequence. Therefore, OSF was followed by the median filter, thus, only the median spectrum was quantified.

Figure 1.16. Comparison between the standard technique of averaging the spectra and OSF. The comparison was performed using a typical measurement with induced artifacts (only showing OFF-spectra). (A) The standard approach of averaging all the acquisitions. The subject movement artifacts are clearly visible in the dynamics M₁₁, M₁₅ and M₂₉. (B) The output from the OSF technique performed on the same dataset as in (A). The median spectrum is computed from the output (O₁₉ and O₂₁) and is used in the quantification. (Reproduced from Tapper S, Tisell A, Lundberg P. (2017) How does motion affect GABA-measurements? Order statistic filtering compared to conventional analysis of MEGA-PRESS MRS. *PLoS ONE* 12(5): e0177795.)

1.8.2 Jackknifing

The jackknife resampling technique is a method for estimating the variance and bias of a dataset. This technique was first introduced by Quenouille in 1949, and later refined by Tukey in 1956 [66, 67]. In addition, the jackknife estimator is a linear approximation of the more famous bootstrap technique. This jackknife estimator is calculated by systematically leaving out one observation from a set of data, then computing an estimation using the remaining observations, and finally averaging over all estimations.

1.8.3 Correlation of Spectral Windows

Wiegiers *et al.* used correlation of spectral windows to perform simultaneous phase and frequency correction of edited MRS data in order to reduce the subtraction artifacts in the difference spectra [68]. Their proposed method performed equally well as spectral registration [69].

1.9 Functional Magnetic Resonance Imaging

When an area of the brain is in use, there is a resulting increased blood flow to that particular region. This blood flow can be detected by functional Magnetic Resonance Imaging (fMRI), which measures brain activity by detecting differences associated with this blood flow [70]. The primary form of fMRI detects the blood-oxygen-level-dependent (BOLD) response, which can be detected either during a task/stimulus or during a resting state. In the latter, the default mode network (DMN) is usually studied. Furthermore, several studies have shown associations between the BOLD response and MRS neurotransmitter concentrations [71-76]. One study has shown an association between the negative BOLD response and the GABA concentration in DMN [74]. Finally, it is also possible to combine the fMRI measurement with simultaneous electroencephalogram (EEG); however, this simultaneous acquisition needs to account for interactions between the EEG equipment and the magnetic field.

2. AIMS

The overall aim of this thesis was to implement, investigate, and evaluate, techniques for GABA detection using MRS in terms of acquisition, post-processing, and final metabolite quantification, and to apply these techniques to clinical populations. The specific aims of this thesis were divided into two sections, ‘applications’ and ‘method development’. First, it was planned that three different clinical populations (narcolepsy, IBS, and ET) would be investigated with single-voxel MEGA-PRESS MRS measurements using conventional post-processing and quantification. Second, due to the challenges when performing clinical MRS single-voxel GABA measurements, the aims of the method development were to develop and investigate methods for retrospective artifact detection and elimination, and to implement and evaluate a pulse sequence intended for fast MRSI GABA detection with full brain coverage.

2.1 Specific Aims of Each Contribution

2.1.1 Applications

- I. The primary aim of paper I was to investigate brain activity changes related to the performance of a verbal working memory task using simultaneous fMRI and EEG, and to correlate these to MRS-quantified levels of GABA and glutamate, in a population of adolescents with narcolepsy. The secondary aim was to investigate the self-reported cognitive issues concerning working memory, and determine whether narcolepsy is characterized by a true working memory deficit, or if it is an imbalance of cognitive resources.
- II. In this combined MRS and resting state fMRI study, the primary aim was to investigate medial prefrontal cortex (mPFC) GABA+ and Glx concentrations and their relation to psychological symptoms in female IBS patients and age-matched healthy controls. Furthermore, using subgroup analyses, the secondary aim was to investigate whether potential biochemical alterations were most pronounced in patients with a severe level of anxiety or depression.
- III. The primary aim of this study was to evaluate the relationship between cerebellar/thalamic glutamate (Glu, determined as Glx) and GABA (determined as GABA+) concentrations, in patients diagnosed with severe essential tremor immediately prior to DBS intervention, and to compare these patients with a healthy control group. The secondary aim was to investigate the relationships between neurotransmitter concentrations and tremor severity, in order to facilitate further conclusions with respect to the underlying pathology, relation to debilitating severity, and metabolic alterations.

2.1.2 Method Development

- IV. In paper IV, the primary aim was to combine cerebellar GABA+ quantification with retrospective artifact reduction using OSF to explore the overall reliability of the computed GABA+ concentrations when intentional subject head movements influenced the MRS data. The secondary aims were to investigate two different approaches to when in the post-processing sequence the filtering should be applied in order to obtain reliable resulting concentration estimates (GABA+, tCr, tNA, and Glx), and to compare these two approaches to the conventional analysis technique.
- V. Closely related to paper IV, the primary aim of this study was to develop a methodology based on an approach of combining jackknifing and correlation of spectral windows (JKC), used for retrospective artifact detection of MRS data. The secondary aim was to investigate the resulting metabolite concentrations (GABA+, tCho, tCr, tNA, and Glx), obtained after the elimination of spectra characterized as artifact-contaminated by the JKC methodology, and compare these to the acquired concentrations when the conventional analysis technique was used.
- VI. In paper VI, the aim was to develop, implement and evaluate an nD spiral-detection MRSI MEGA-sLASER sequence developed for a conventional clinical MR-scanner, and to identify the most prominent challenges in the implementation.

3. MATERIALS AND METHODS

3.1 Patients and Volunteers

This section describes the study participants recruited and investigated in each paper included in this thesis. First, three clinical populations and corresponding healthy controls are described; narcolepsy (paper I), irritable bowel syndrome (paper II), and essential tremor (paper III). Second, a group of healthy volunteers was recruited for the purpose of developing and investigating retrospective approaches (OSF and JKC) for detection and elimination of artifacts in MRS data (paper IV and V). Finally, only phantom measurements were performed using the nD MRSI sequence (paper VI).

3.1.1 Narcolepsy Patients and Healthy Controls

In the narcolepsy study (SAND:MAN), 19 adolescent participants (13-19.5 years) with a confirmed diagnosis of narcolepsy were recruited from a population-based study in western Sweden, as well as pediatric clinics in Östergötland county. The patients met the criteria for type 1 narcolepsy [77], apart from one patient who did not have cataplexy and lacked measurement of CSF-hypocretin. Additionally, 21 healthy controls (13.1-24.1 years) were recruited by advertisement to match the age and gender distribution of the narcolepsy patients. All healthy controls were confirmed to have no medical history of mental illness or neurological disorders prior to inclusion in this study.

3.1.2 IBS Patients and Healthy Controls

A total of 64 right-handed females (31.6 ± 8.8 years) with a confirmed diagnosis of IBS based on the Rome III diagnostic criteria, and 32 right-handed healthy females (34.2 ± 10.7 years) were included in this study. The patients were referred to the gastrointestinal unit of the Linköping University Hospital, and the healthy controls were recruited by local advertisement. Moreover, patients with celiac disease or inflammatory bowel disease were excluded after undergoing a standard clinical examination. Additionally, other exclusion criteria were metabolic disorders, neurological disorders, severe psychiatric disorders, smoking, claustrophobia, large metal implants, pacemaker, or large tattoos. Finally, for the healthy controls, a medical history of gastrointestinal symptoms or psychiatric disorders, or the usage of a centrally-acting medication were also exclusionary.

3.1.3 ET Patients and Healthy Controls

Ten right-handed essential tremor patients (60.2 ± 9.7 years, seven males) that were referred to the neurosurgical department in Linköping after being assessed and diagnosed by a movement disorder specialist were included in this study. Due to the disabling tremor severity of the upper limb (most symptomatic on the right side), all patients in this study were offered DBS surgery. Only one patient declined the surgery, and the rest of the patients later underwent successful DBS intervention. Prior to surgery, all patients were evaluated by a neuropsychologist, and no psychiatric or cognitive impairment was found in any of the patients. Also, no other signs of neurological disorders other than ET were found in the patient group. Furthermore, during the time of examination, none of the patients were treated with medications known to induce tremor or affect GABA pathways (*e.g.* Primidone, clonazepam, gabapentin or phenobarbital) [78]. Finally, six right-handed matched healthy controls (62.2 ± 11.4 years, five males) without any medical history of neurological illness were recruited by local advertising.

3.1.4 Healthy Volunteers

For the purpose of collecting MRS data with and without motion-induced artifacts, 12 healthy volunteers (29.5 ± 11.0 years, four males) were recruited by local advertisement. The resulting MRS data were used in both papers IV and V, which investigated retrospective approaches for more reliable metabolite quantification when artifacts were present in the data.

3.2 Phantoms

Two different phantoms (Table 3.1) were used in the MRSI measurements in paper VI.

- Braino is a spherical spectroscopy phantom (GE MRS-HD-Sphere, General Electric, Milwaukee, USA), which is commonly used when implementing and exploring new MRS applications. Braino has a diameter of approximately 17 cm, and a 7.20 ± 0.05 pH.
- 'STO-GABA' is a 'cube-in-sphere' phantom (STO-01, [79]; manufactured by Prototal AB, Jönköping, Sweden) that consists of a $5 \times 5 \times 5$ cm³ cube filled with an aqueous saline solution containing GABA and creatine, which is centered inside a spherical compartment filled with saline.

Table 3.1. Description of the two phantoms. Concentrations in mM of the most relevant metabolites in the Braino phantom (for further information see [80]), and the metabolites included in the STO-GABA phantom.

Metabolite	Braino [mM]	STO-GABA [mM]
Cho	3.0	N/A
Cr	10.0	20.0 (inner cube)
GABA	N/A	30.0 (inner cube)
Glu	12.5	N/A
Lac	5.0	N/A
ml	7.5	N/A
NAA	12.5	N/A
NaCl	N/A	150.0 (sphere)

Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.

3.3 Pulse Sequences

Two different pulse sequences were used for the purpose of collecting the MRS data used in this thesis.

3.3.1 Single-Voxel MRS MEGA-PRESS

The pulse sequence used for single-voxel MEGA-PRESS MRS was distributed by Professor Richard Edden (Johns Hopkins University). This sequence was based on the regular single-voxel PRESS experiment, but with two added frequency-selective MEGA editing pulses (as described in the introduction section).

3.3.2 MRSI Sequence

The MEGA-sLASER version of the pulse sequence that was developed in collaboration with GyroTools LLC (Zürich, Switzerland) is illustrated in Figure 3.1. This sequence was developed for a conventional clinical Philips Ingenia MR system (Philips Healthcare, Best, the Netherlands). Moreover, this sequence was inspired by the 3D MEGA-LASER sequence with which Bogner *et al.* (2014) have reported very promising results for GABA detection [61].

One of the main purposes of this MRSI pulse sequence was to use adiabatic localization in order to compensate for the CSDE. Moreover, the sLASER localization was accomplished by a non-adiabatic slice-selective 90° excitation pulse (9.7 ms duration, 13.5 μ T B₁ max), followed by two pairs of adiabatic hyperbolic secant refocusing pulses (4.4 ms duration, 13.5 μ T B₁ max). Additionally, for comparison purposes, standard PRESS localization was

also implemented in which a slice-selective 90° excitation pulse was followed by two non-adiabatic slice-selective 180° refocusing pulses.

For the purpose of GABA detection, MEGA editing could be enabled, and performed by two Gaussian refocusing pulses (14.0 ms duration) positioned in the sequence as shown in Figure 3.1. To have time to utilize localization, gradient spoiling and editing, it was necessary to have a slightly longer echo time (TE = 80 ms). Finally, the editing pulses were surrounded by spoiler gradients (2.2 ms duration, 22.5 mT/m amplitude), which were arranged as proposed by Mescher *et al.* (1998) [54] and later used by Bogner *et al.* (2014) [61].

The total acquisition time was minimized by using a fast readout utilized with a set of constant density spirals in the x-y plane. Additionally, in 3D measurements, the readout would be performed by acquiring a stack of spirals by using phase encoding superimposed over the last MEGA-spoiler.

Figure 3.1. Pulse-diagram of the MRSI MEGA-sLASER pulse sequence. The sLASER localization consists of a non-adiabatic slice-selective excitation followed by two pairs of adiabatic selective refocusing pulses. MEGA-editing is enabled for the purpose of GABA detection. Finally, spiral readout is used for a fast data acquisition. The adiabatic refocusing pulses are indicated by the light blue blocks, and the light yellow blocks indicate the MEGA spoiler gradients. (Reproduced from Tapper *et al.*, 2019, *nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges*, in manuscript).

3.4 Data Acquisitions

The data acquisitions in this thesis were performed according to the timeline in Figure 3.2. In all acquisitions, a 3 T Philips Ingenia MR system (MR3 or MR5, Philips Healthcare, Best, Netherlands) was used. As indicated in this figure, it is important to note that we received a new identical MR system (MR5) in the

summer of 2015, and the IBS study was particularly affected by this change of system. Furthermore, not only MRI measurements were described in this section; clinical measurements were also described where applicable.

Figure 3.2. Timeline of the measurements. Timeline diagram showing when the data acquisitions were performed in each study/project. During the summer 2015 (red line), we received a new identical MR system, thus, both MR3 and MR5 were 3 T Philips Ingenia MR systems.

3.4.1 Single-Voxel MEGA-PRESS Measurements

All single-voxel MEGA-PRESS measurements were performed with the ON/OFF editing pulses at 1.90/7.46 ppm, and using a TR/TE = 2000/68 ms. Moreover, a total of 40 dynamics were collected in each measurement using eight phase cycle steps, thus producing a total of 320 excitations. Additionally, for the purpose of weak water suppression, MOIST water suppression was performed. Directly after each water suppressed MEGA-PRESS measurement, an identical but shorter (two dynamics) unsuppressed water MEGA-PRESS measurement was acquired to obtain a reference of water within the tissue in the voxel.

3.4.2 fMRI and MRS MEGA-PRESS in Narcolepsy

In the narcolepsy study, all participants were scanned using MR3 equipped with a 32-channel phased-array head coil (Figure 3.3). First, T_1 weighted images were acquired to ensure that the participants were clear of obvious pathological abnormalities. Second, the fMRI data were acquired using a single-shot gradient-echo EPI sequence, which covered the whole brain in 2.2 s. During the working memory task, the participants were instructed to read sentences and decide whether each one made sense [81]. A total of 18 sequences of sentences were presented in a pseudo-random order in an event-related fMRI design (total time of 12 min 45 s). Additionally, the participants were instructed to memorize the last word of each sentence. After the presentation of a sentence block, the participants were presented with four words (one at a time, for 1 s), and were instructed to press Yes or No if the word had been presented

in the preceding sentence block. During the fMRI working memory task, a simultaneous EEG was also acquired. Finally, following fMRI, a MEGA-PRESS MRS measurement was acquired with the voxel ($3 \times 3 \times 3 \text{ cm}^3$) placed in the medial prefrontal cortex (mPFC).

Figure 3.3. *MR protocol used in the narcolepsy study. Note that the fMRI acquisition was performed prior to MRS.*

3.4.3 MRS MEGA-PRESS, fMRI and Questionnaires in IBS

In the IBS study, all participants were scanned using a Philips Ingenia MR system equipped with a 32-channel phased array head coil (Figure 3.4). However, as previously mentioned, in the middle of the data acquisitions, we switched from MR3 to MR5, which were “identical” MR systems.

First, structural T_1 weighted images were collected to exclude brain abnormalities and to ensure correct voxel placement in following the MRS measurement. Second, MEGA-PRESS MRS was acquired with the voxel ($3 \times 3 \times 3 \text{ cm}^3$) placed in the mPFC according to Figure 3.5. After MRS, an eyes-closed resting state fMRI was performed using a single-shot gradient-echo EPI sequence, which covered the whole brain.

Figure 3.4. *MR protocol used in the IBS study. In contrast to the narcolepsy study, the MRS measurement were performed prior to fMRI.*

3.4.3.1 Questionnaires

All participants completed the four following questionnaires to assess individual clinical measurements:

- HADS was used to assess symptoms of anxiety and depression.
- VSI was used to measure GI symptom-specific anxiety.
- IBS-SSS was used to evaluate the severity of the IBS and the interference with life.
- BPI was used to assess pain intensity and interference with functional and emotional domains.

Figure 3.5. Typical voxel placement used in the data acquisitions in the narcolepsy and IBS studies. The voxel ($3 \times 3 \times 3 \text{ cm}^3$, indicated by the red box) was placed in the medial prefrontal cortex. (A/P = anterior/posterior, H/F = head/feet, L/R = left/right)

3.4.4 MRS MEGA-PRESS in ET

All participants were scanned using MR3 equipped with a 12-channel phased array head coil. First, structural T₂ weighted images were acquired (Figure 3.6) to ensure correct voxel placement in the MRS measurements. Moreover, four MEGA-PRESS measurements were performed with two voxels ($3 \times 3 \times 3 \text{ cm}^3$) placed in the left and right thalamic regions, and two voxels ($3.5 \times 2.5 \times 2.5 \text{ cm}^3$) placed in the left and right cerebellar regions according to Figure 3.7.

3.4.4.1 Estimating Tremor Severity

The Essential Tremor Rating Scale (ETRS) was used according to Fahn, Tolosa, and Marin, to evaluate the tremor severity of the participants. Moreover, only the right upper extremity was evaluated with part A item 5, and part B items 10-14. The tremor severity was defined by the sum score of these items, with 32 as the maximal score.

MR Protocol

Figure 3.6. MR protocol used in the ET study. Four different voxel placements were investigated.

Figure 3.7. Typical voxel placements used in the data acquisitions in the ET study, only showing the left placement. A $3 \times 3 \times 3 \text{ cm}^3$ voxel was placed in the left and right thalamic region, and a $3.5 \times 2.5 \times 2.5 \text{ cm}^3$ voxel in the left and right cerebellar region. (R/L = right/left).

3.4.5 Method Development: Healthy Volunteers

All volunteers were scanned using MR5 equipped with a 12-channel phased-array head coil. In the data acquisitions, the measurement protocol (Figure 3.8) consisted of two MEGA-PRESS measurements acquired with the voxel ($3.5 \times 2.5 \times 2.5 \text{ cm}^3$) placed in the left cerebellar region as shown in Figure 3.9. This specific voxel placement was inspired by one of the voxel placements used in the ET study. Furthermore, the first MEGA-PRESS measurement did not include any intentional subject head movements, and was thus considered as a reference measurement. Meanwhile, the second MEGA-PRESS measurement was identical in terms of acquisition parameters but included four episodes of intentional head movements. In addition, T_2 weighted images were collected before and after the spectroscopy measurements to ensure correct voxel placement. Finally, for each volunteer, the measurement protocol was scanned three times (test-retest).

MR Protocol

Figure 3.8. MR protocol. MR protocol used in the data collections for the purpose of investigating the retrospective techniques for artifact reduction, detection and elimination.

3.4.5.1 Movement Paradigm

The movement paradigm, thus the starting excitation and duration of each of the movement episodes, was randomized using MATLAB (MathWorks, Natick MA), in which the excitations could be between two to eight excitations long. Each volunteer was instructed to move either in the left-right (“yaw”) or the up-down (“pitch”) direction, and to find the original head placement after each episode. The paradigm was conducted following commands given through the communication speakers in the scanner.

Figure 3.9. Typical voxel placement used in the data acquisitions. The voxel ($3.5 \times 2.5 \times 2.5 \text{ cm}^3$, indicated by the yellow box) was placed in the left cerebellar region in all volunteers. (A/P = anterior/posterior, R/L = right/left)

3.4.6 MRSI Acquisitions

All MRSI measurements were performed on MR5 equipped with either a 12-channel phased array head coil ($13.5 \mu\text{T}$ B_1 max) or a transmit-receive (T/R) head coil ($20 \mu\text{T}$ B_1 max). Following imaging to ensure correct VOI placement (Volume of Interest), water suppressed MRSI (VAPOR, 140 Hz bandwidth) was performed, in each case followed by a shorter identical (two averages) unsuppressed water MRSI measurement to obtain a reference of water from within the VOI. Furthermore, only 2D measurements were performed in this thesis, and Table 3.2 summarizes the protocol parameters that were used throughout all the MRSI measurements.

Table 3.2. Acquisition parameters. Acquisition parameters that were used during all the phantom measurements performed. (TR = repetition time, FOV = field of view).

Parameters		Spiral Readout	
TR [ms]	1600	N _{ang}	4
TE [ms]	80	N _{temp}	2
FOV [mm ²]	200 x 200	Spirals/TR	300
Voxel size [mm ³]	10 x 10 x 10	Samples/spiral	64
Slice thickness [mm]	10	Spiral duration [ms]	1.5
Spectral width	1000	Recon matrix	20 x 20
Phase cycles	1		

Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.

3.4.6.1 Braino Measurements

The protocol parameters that were changed between the Braino measurements are described in Table 3.3. Moreover, a total of eight MRSI measurements using PRESS, sLASER, MEGA-PRESS, and MEGA-sLASER, were performed with the VOI (100 x 100 mm²) centered in Braino. When using eight averages, the total acquisition time was 1 min 45 s. In addition, a longer MEGA-sLASER MRSI measurement (80 averages, approximately 17 min) was performed when using the T/R head coil.

Table 3.3. Overview of the nine measurements performed using Braino. Localization technique, if MEGA-editing was enabled, the number of averages, and finally which head coil, that were used during these measurements.

Measurement	Localization	MEGA-edit	Averages	Coil
I	PRESS	No	8	12ch
II	sLASER	No	8	12ch
III	PRESS	Yes	8	12ch
IV	sLASER	Yes	8	12ch
V	PRESS	No	8	T/R
VI	sLASER	No	8	T/R
VII	PRESS	Yes	8	T/R
VIII	sLASER	Yes	8	T/R
IX	sLASER	Yes	80	T/R

Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.

3.4.6.2 STO-GABA Measurements

Table 3.4 summarizes the protocol settings used in the STO-GABA measurements. Five measurements were performed using the T/R head coil with the VOI (100 x 100 mm² or 200 x 200 mm²) centered in the STO-GABA. Furthermore, as for Braino, a longer MEGA-sLASER MRSI measurement (80 averages, approximately 17 min) was also performed for the STO-GABA. Finally, for comparison purposes, a conventional single-voxel MEGA-PRESS measurement was performed with the voxel centered in the inner cube of the STO-GABA.

To investigate the effect a limited B₁ amplitude has on the sLASER localization, eight unsuppressed water MRSI measurements were performed with the VOI (100 x 100 mm²) centered in the STO-GABA. Since the T/R head coil was used in these measurements, a maximal B₁ amplitude of 20 μ T was achieved. Therefore, we performed the first measurement with a B₁ max at 20 μ T, and then the MRSI measurement was repeated but with a 1 μ T decrease in B₁ max each time. Moreover, this was continued until a maximal amplitude of 13 μ T was achieved, which was comparable with the maximal available amplitude in the 12-channel head coil.

Table 3.4. The five MRSI measurement performed on STO-GABA. Localization technique, if MEGA-editing was enabled, the number of averages, and the VOI size used during these five measurements.

Measurement	Localization	MEGA-edit	Averages	VOI [mm ²]
I	PRESS	Yes	8	100 x 100
II	sLASER	Yes	8	100 x 100
III	PRESS	Yes	8	200 x 200
IV	sLASER	Yes	8	200 x 200
V	sLASER	Yes	80	100 x 100

Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.

3.5 Data Processing and Analysis of Clinical MRS Data

The single-voxel MEGA-PRESS data collected in the clinical applications were post-processed and analyzed using the same approach. Since these data were collected using a Philips system, the export data format was SPAR/SDAT, which is a format that includes each dynamic average. The FIDs from the dynamic averages were individually phase corrected according to Klose [82] and frequency aligned based on the water residual [83]. For the purpose of GABA+ detection, the OFF dynamics were subtracted from the corresponding ON dynamic, prior to averaging the difference spectra. The resulting averaged difference spectrum was used as input to LCModel (Ver 6.3-1E for the narcolepsy data, Ver 6.3-1L for the IBS and ET data) [63], and the GABA+ concentration was extracted from each dataset. Furthermore, the corresponding Glu or Glx concentration was computed using only the averaged OFF spectra as input to LCModel. Moreover, two different basis sets were used in the LCModel quantification. First, in the analyses of the narcolepsy data, the basis sets were obtained from Dr. S. Provencher, developer of LCModel. Second, in the analyses of the IBS and ET data, the basis sets were obtained from the Dydak Lab [53, 84, 85]. In addition, all concentrations were water-scaled using the separately collected water reference (DOWS = T), with the purpose of obtaining the resulting concentrations in units of mM. Finally, Figure 3.10 shows representative OFF and GABA difference spectra, fitted using LCModel.

Figure 3.10. Representative OFF spectrum (left) and difference spectrum (right), fitted using LCModel. The OFF spectrum was used for Glu/Glx extraction, and the difference spectrum was analyzed with the purpose of GABA+ quantification. Assignments: 1, Creatine ($-^2\text{CH}_2-$); 2, Glx ($-^2\text{CH}-$); 3, Choline ($-N(\text{CH}_3)_3$); 4, Creatine ($-N(\text{CH}_3)_3$); 5, GABA+ ($-^4\text{CH}_2-$); 6, tNA ($-^3\text{CH}_2-$); 7, Glx ($-^4\text{CH}_2-$); 8, GABA+ ($-^2\text{CH}_2-$); 9, tNA ($-^2\text{CH}_3$); 10, GABA+ ($-^3\text{CH}_2-$); 11-13, Macromolecules and lipids, $-^2\text{CH}_2-$.[53]

3.6 What is Raw Data?

In the method development, it was necessary to obtain the spectrum from each single voxel excitation. These raw data (lab-file and raw-file formats) consist of many more data points than the typically exported formats SPAR/SDAT on the Philips platform. These raw data correspond to the completely unprocessed measurement data, which are the output from the analog-to-digital converter. Therefore, from these raw data, it was possible to obtain the spectrum from each individual excitation, thus the spectrum from each phase cycle step, ON/OFF dynamics, and separate coil element. Since the readout time was 512 ms in duration, the number of samples $N_{\text{raw}} = 16384$, as the sampling frequency was 32 kHz in the raw data. Furthermore, these raw datasets were reconstructed using ReconFrame (GyroTools LLC, Zürich, Switzerland), which is a MATLAB-based software package used for offline reconstruction of MR data. This reconstruction generated the FIDs from each ON/OFF dynamic, phase cycle step, and coil element.

The raw data from the MRSI acquisition were also exported due to the necessity for offline reconstruction of the spirals. Here, Pack 'n Go (GyroTools LLC, Zürich, Switzerland) was used to extract the raw data from the scanner. Prior to reconstruction, each raw dataset consisted of the spiral profiles from each angular and temporal interleave, coil element, and signal average (NSA). Moreover, since all measurements were performed using 64 samples/spiral and the scanner sampling frequency was 32 kHz, each k-space profile consisted of 2048 samples. After reconstruction of the 2D MRSI data, the resulting data size was [spirals/excitation x N_{temp}] x size of the reconstruction matrix x coil elements x NSA.

3.7 Data Processing and Analysis of Volunteer MRS Data

Following reconstruction, phase correction according to Klose [82] and frequency alignment based on the water residual [83], then, coil combination using SNR weighting based on the water signal from the water reference measurement was performed. At this point, the resulting data size was phase cycle steps $\times$ dynamics $\times$ samples ($P \times M \times N_{\text{raw}}$). Due to the differences in spectral appearance between ON and OFF spectra, these were handled separately in both OSF and JKC.

3.7.1 Order Statistic Filtering

As shown by Figure 3.11, three different schemes were investigated using each of the post-processed motion-influenced or reference MRS datasets. Two different approaches using OSF were used ('RAW PC OSF' and 'RAW Dyn OSF'), depending on when the rank-ordering was applied. In the RAW PC scheme, the rank-ordering and median filtering were applied to the individual phase cycle steps prior to averaging. However, with the RAW Dyn OSF scheme, the rank-ordering and median filtering were applied to the dynamics after averaging of the phase cycle steps. For comparison purposes, the standard technique was applied as one scheme ('RAW Standard'), thus, the resulting spectra were computed using standard averaging of the phase cycle steps and the dynamics.

For all three schemes, the resulting spectrum was computed by subtracting the resulting OFF spectrum from the resulting ON spectrum, which generated three resulting spectra for each dataset. These difference spectra were quantified using LCModel (Ver 6.3-1E) [63] using basis sets obtained from the Dydak Lab [53, 84, 85], which generated three distinctive GABA+ estimates for each dataset. Furthermore, the same procedure was used again for solely the OFF data, which for each dataset generated three distinctive estimates of tCr, tNA, and Glx. Finally, all concentrations were water-scaled using the separate water reference measurement.

Figure 3.11. The workflow over the three different schemes. These three different schemes were used for post-processing the MEGA-PRESS data obtained from the acquisitions, regardless of whether the measurement contained movements. The OSF was performed in two different ways; rank-ordering the phase cycles 'OSF PC' and rank-ordering the dynamics 'OSF Dyn'.

3.7.2 Jackknife Correlation

Figure 3.12 illustrates the workflow for the whole process from data acquisition to quantification when JKC was implemented and validated. After reconstruction, phase and frequency correction, and combination of coil elements, the data were split into ON and OFF excitations and thereafter sorted according to phase cycle step. Furthermore, the motion-influenced datasets from protocol 1 were used as training data for the implementation of an optimal JKC methodology, and the reference datasets from protocol 1 and the datasets from protocols 2-3 were used for validation.

Figure 3.12. Workflow using JKC. The different processing steps performed during the pre-processing, JKC implementation and validation, and post-processing, before the metabolite quantification using LCModel. (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med.* 2018;00:1-15)

3.7.2.1 Correlation of Spectral Windows

As shown in Figure 3.13, two different spectral windows were investigated in the correlation analyses. Consequently, only spectral points included within these two windows were used in the correlation analyses. First, the metabolite window corresponded to the analysis window (1.0 – 4.0 ppm), and second, the water window corresponded to the 140 Hz bandwidth of the MOIST water suppression (4.15 – 5.25 ppm).

Figure 3.13. *The two spectral windows used in the correlation analyses. The water window (blue) corresponded to the bandwidth of the MOIST water suppression (4.15 – 5.25 ppm), and the metabolite window (red) corresponded to the analysis window used in the quantification (1.0 – 4.0 ppm).*

Furthermore, one correlation procedure was applied separately to each MRS measurement, each of the eight phase cycle steps, and each type of ON/OFF editing. Then, as shown by Figure 3.14, for each of the M extracted comparable spectral windows, the spectral window was correlated (Pearson) to the mean spectral window computed by averaging the other M-1 spectral windows. Thus, a leave-out-one analytical approach was used, which is the core of jackknifing. This process was repeated until all M spectral windows had been correlated to the mean of the others, which in total generated M correlation coefficients. This procedure was in turn repeated for each of the eight phase cycle steps and ON/OFF-edited data, which in total generated an $M \times 16$ matrix for each dataset that contained all resulting correlation coefficients.

Figure 3.14. Correlation analyses performed using JKC. Example of how the correlation analyses were conducted and illustrated for one phase cycle step in the OFF data from one dataset. First, the green spectral window was correlated to the mean of the rest M-1 spectral windows. This procedure was repeated M times, and each time, the resulting correlation coefficient was save to a matrix, which here was illustrated by color-coding according to the color of the spectra used.

3.7.2.2 Finding Optimal Cutoff

After the correlation analyses of the training data, the next step was to find the optimal cutoff value of the correlation coefficient. The Youden index [86] was used to find this optimal cutoff, which determined under what value of the correlation coefficient the corresponding spectra should be discarded and thus eliminated from further analysis. Moreover, the Youden index is the cutoff for where the sensitivity and specificity are maximized, and here, a true positive was defined as a correctly identified movement-influenced spectrum according to the movement paradigm. A total of four optimal cutoffs were computed, one for OFF and ON, and using both spectral windows.

3.7.2.3 Quantification

After discarding all spectra (excitations) that gave a resulting correlation coefficient under the cutoff, the rest of the spectra were averaged (ON and OFF spectra separately) and down-sampled to 16 kHz. Finally, the quantification was performed using Ver 6.3-1L of LCModel, and in contrast to the evaluation of OSF, total choline (tCho) was also extracted and investigated here.

3.8 Processing and Analysis of MRSI Data

When the MRSI data were analyzed, the specific post-processing and analysis steps varied depending on the parameter settings that were used in the acquisition, and also, on the purpose of the measurement being analyzed (summarized by Figure 3.15). ReconFrame was used for reading, reconstructing, processing, and finally writing to SPAR/SDAR file formats readable by LCModel. Moreover, when the 12-channel phased array coil was used, the coil combination was performed using SNR weighting. Note that this coil combination was performed outside of ReconFrame.

3.8.1 Quantification

Each voxel in the MRSI datasets was quantified, and concentration maps that corresponded to the 20 x 20 reconstructed grid of voxels were computed.

3.8.1.1 LCModel

The metabolite quantification was performed using LCModel [63] (Ver 6.3-1L). Eddy current correction was performed based on the water reference (LCModel parameter DOECC = T); however, no water scaling was performed (DOWS = F). Moreover, since phantom measurements were analyzed, the VITRO parameter was set to T. Also, in the analyses of the STO-GABA phantom data, additional parameters needed to be modified as there was no strong conventional metabolite such as NAA, Cr, or Cho in the MEGA-edited spectra (LCModel parameters NCALIB = 1, CHCALI = GABA).

3.8.1.2 Fitting the Water Signal

When applicable, the water signal was quantified by fitting a Lorentzian line-shape to the water signal at 4.68 ppm in the water reference dataset. Prior to fitting, the data were Klose-corrected [82] to remove eddy currents, zero-filled and line-broadened (LB = 3 Hz). Finally, the area under the fitted curve was considered to be proportional to the water concentration.

Figure 3.15. Work-flow showing each step performed from data acquisition to computing the resulting concentration maps. The “A” shows where the data were extracted for the purpose of investigating the drifts in K0, not every dataset was investigated, but some characteristic datasets were chosen. The black box indicates all functions that were utilized from ReconFrame. Note that the coil combination was performed outside of ReconFrame.

3.9 Statistical Analyses of MRS Concentrations

3.9.1 Narcolepsy Patients Compared with Healthy Controls

GABA+ and Glu concentrations were compared between the narcolepsy patients and the healthy control group. Also, Pearson correlation analyses were performed of the concentrations and the working memory task-related activation extracted from 8 mm spherical regions of interest placed at the stereotactic peaks of activation and deactivation in the mPFC. Then, a Fisher's Z transformation was used to test for between-group differences in the resulting correlation coefficients. For the correlation analyses, significance was assessed at $p < 0.006$ since Bonferroni correction was performed for compensating for the eight separate between-group tests.

3.9.2 IBS Patients Compared with Healthy Controls

Since a Shapiro-Wilk test revealed non-normal distribution of questionnaire data in the healthy controls, a non-parametric Mann-Whitney U-test and Spearman correlation analyses were performed to detect any differences between the IBS patients and the healthy controls. Furthermore, to investigate potential relationships between neurotransmitter concentrations and the severity of psychological symptoms in IBS, patient subgroups were defined according to HADS scores, with a score of ≥ 11 as a well-established cutoff for the detection of anxiety or depression [87, 88]. These between-group analyses were performed using the Kruskal-Wallis test followed by post-hoc U-tests with Bonferroni correction. Finally, the statistical analyses were performed using the IBS SPSS Statistics 25 software (IBM Corporation, Armonk, NY, USA), and the significance level was set at $p < 0.05$.

3.9.3 ET Patients Compared with Healthy Controls

A non-parametric approach was used in the statistical analyses, since the ET patients were not considered to be normally distributed. Both cerebellar and thalamic MRS were acquired from the left and right hemispheres, which were analyzed separately, but also combined as a bilateral concentration by averaging the left and right concentration estimates to increase the power of the measurement. Furthermore, the GABA+, Glx and GABA+/Glx ratio was investigated between the ET patient group and the healthy controls by computing unpaired non-parametric Mann-Whitney tests. In addition, due to previous reported cerebellar GABA+ asymmetry in ET [78], the Mann-Whitney test was also used to investigate potential GABA+ asymmetry in the ET patient group. Furthermore, analyses were also performed to correlate the GABA+ and Glx concentrations, and the GABA+/Glx ratio, to the ETRS score. These correlation analyses were performed using the Spearman correlation and the corresponding p-values were computed to show significance. Finally, a significance level of $p < 0.05$ was chosen for all analyses.

3.9.4 OSF Compared with Standard Averaging

Bland Altman plots [89] and correlation analyses were used to illustrate how the resulting GABA+ and tCr concentrations were distributed using standard averaging and OSF. These plots were also used to illustrate the potential influence of subject movements on the resulting MRS concentrations.

Furthermore, paired t-tests were used to detect significant differences in the computed concentrations between the three schemes using the same datasets. In addition, paired t-tests were also performed to detect differences in computed concentrations between the motion influence measurement and the corresponding reference measurement, which originated from the same measurement protocol. To exclude the influence from motion, paired t-tests were also used to detect any differences in the computed metabolite concentrations between the different schemes using solely the reference data.

To summarize, a total of 24 paired t-tests were performed; six for each metabolite (GABA+, tCr, tNA, Glx). Three of these detected differences between the schemes and three detected differences due to motion artifacts. Finally, the corresponding 95% confidence intervals were also computed, and a significance level of $p < 0.05$ was chosen in all tests.

3.9.5 JKC Compared with Standard Averaging

Three paired t-tests were performed for each metabolite concentration (tCho, tCr, tNA, GABA+, Glx), with the purpose of investigating the differences between the methodological approaches. First, JKC was compared with standard averaging when using the reference data. Second, using the standard averaging method, the references were compared with the measurements containing movement artifacts. Third, the same as the second comparison, but instead using the implemented JKC method. As when investigating OSF, the corresponding 95% confidence intervals were also computed, and a significance level of $p < 0.05$ was chosen for all comparisons.

4. RESULTS

4.1 mPFC GABA+ and Glu in Narcolepsy

No significant differences were observed in mPFC GABA+ or Glu between the narcolepsy patients and the healthy controls (Table 4.1). In addition, the correlation analyses between GABA+ and Glu concentrations and the relative BOLD deactivation in the mPFC during the encoding of sentences resulted in trend-level p-values. All other correlations were non-significant. Furthermore, during the encoding of sentences, the narcolepsy patients and healthy controls had opposite patterns of correlation for both GABA+ and Glu, while during the recognition of words, both groups exhibited the same pattern of correlation for both metabolites (Figure 4.1).

Table 4.1. Results from analysis of the GABA+ and Glutamate MRS data. The table shows both the relative concentrations of each metabolite for each group, as well as, the correlation coefficients (Pearson) between each metabolite concentration and peak areas of task-related activation and deactivation in the medial prefrontal cortex for both the encoding of sentences and the recognition of words.

Average Concentration	Narcolepsy	HC	T	p
GABA+	0.85 ± 0.14	0.83 ± 0.21	0.45	n.s.
Glutamate	5.1 ± 0.86	5.3 ± 1.1	0.68	n.s.
Correlation Coefficients	Narcolepsy	HC	Z	p
Encoding of Sentences				
GABA+ and mPFC activation	0.47	-0.05	1.4	n.s.
GABA+ and mPFC deactivation	0.17	-0.38	1.5	0.1
Glu and mPFC activation	-0.11	0.29	1.0	n.s.
Glu and mPFC deactivation	-0.42	0.26	1.8	0.07
Recognition of Words				
GABA+ and mPFC activation	0.31	0.20	0.3	n.s.
GABA+ and mPFC deactivation	-0.30	-0.40	0.3	n.s.
Glu and mPFC activation	0.24	0.30	0.1	n.s.
Glu and mPFC deactivation	0.14	0.40	0.7	n.s.

Figure 4.1. Correlation analyses. Results from correlation analysis comparing GABA+ (A) and Glutamate (B) concentrations to BOLD activity levels in medial prefrontal cortex during the encoding of sentences. (A) GABA+ with deactivation in medial prefrontal cortex (mPFC). (B) Glutamate with deactivation in mPFC. Dashed lines indicate best linear fit, and narcolepsy data points are indicated by light gray diamonds and healthy controls by dark gray squares.

4.2 mPFC GABA+ and Glx in IBS

The between-group comparisons of mPFC neurotransmitter levels revealed no significant differences in GABA+ or Glx concentrations between IBS patients and healthy controls.

4.2.1 mPFC Concentrations Related to Psychological Symptoms

The IBS patients exhibited significantly higher severity of anxiety and symptoms of depression than the healthy controls. In the full sample, GABA+ concentrations were most strongly associated with anxiety symptoms ($p = 0.009$, Figure 4.2 A), and showed a weaker correlation with depression symptoms ($p = 0.044$, Figure 4.2 B). Meanwhile, Glx was not associated with any psychological symptoms. Furthermore, when performing the analyses with the IBS patients and healthy controls separated, a distinct association between GABA+ concentrations and anxiety was observed ($p = 0.019$). However, no significant associations were detected between GABA+ and symptoms of depression in IBS.

4.2.2 IBS Subgroups Based on Anxiety Scores

To further investigate the relationship between anxiety and mPFC GABA+ in IBS, the patients were subdivided into two groups based on low (IBS-) and high (IBS+) severity of anxiety symptoms (Figure 4.3 A). Then, significantly higher GABA+ concentrations were observed in the IBS+ relative to the IBS- subgroup (Figure 4.3 B).

Figure 4.2. mPFC GABA+ related to psychological symptoms. Spearman's rank correlation between mPFC GABA+ concentrations and HADS anxiety scores in all subjects. Patients with IBS ($N = 64$) are depicted as red circles, HCs ($N = 32$) as blue squares. (Reproduced from Icenhour A, Tapper S, Bednarska O, et al. Increased Inhibitory Neurotransmitter Concentration in Medial Prefrontal Cortex is Associated with Anxiety in Irritable Bowel Syndrome. In manuscript).

Figure 4.3. Group analyses. Group differences in HADS anxiety scores (A) and mPFC GABA+ concentrations (B) between HCs ($N = 32$; blue), IBS patients with low severity of anxiety symptoms ($N = 34$; red striped) and patients with high severity of anxiety symptoms ($N = 30$; solid red). Data are given as the median and error bars indicate interquartile ranges. ** $p < 0.01$; *** $p < 0.001$. (Reproduced from Icenhour A, Tapper S, Bednarska O, et al. Increased Inhibitory Neurotransmitter Concentration in Medial Prefrontal Cortex is Associated with Anxiety in Irritable Bowel Syndrome. In manuscript).

4.3 GABA+ and Glx in ET

No significant between-group changes were observed in either bilateral, left or right GABA+ and Glx concentrations, or in the corresponding GABA+/Glx ratios. However, as shown by Figure 4.4, a non-significant higher mean Glx concentration was observed in both cerebellar and thalamic voxel placements for the healthy controls compared to the ET patients.

Figure 4.4. Cerebellar and thalamic concentrations. Mean concentration of GABA+ $\pm$ standard deviation for the left and right voxel placements in cerebellum (A) and thalamus (B) in ET-patients ($n = 10$). (Reproduced from Tapper S, Göransson N, Tisell A, et al. Essential Tremor: Cerebellar GABA+/Glx Ratio is Correlated with Tremor Severity. In manuscript).

4.3.1 Association between GABA+/Glx Ratio and Tremor Severity

The ETRS score indicated the overall high tremor severity of the ET patient group (17.6 ± 2.9). Meanwhile, none of the healthy controls experienced any sign of tremor (ETRS = 0). Furthermore, bilateral cerebellar or thalamic GABA+ and Glx concentrations did not show any significant correlation to ETRS. However, the bilateral cerebellar GABA+/Glx ratio was positively correlated to the ETRS ($p = 0.03$). Figure 4.5 shows the correlation between the left (A) and

right (B) cerebellar GABA+/Glx and ETRS. The right cerebellar GABA+/Glx ratio was still positively correlated to ETRS ($p = 0.02$); meanwhile, only a positive trend ($p = 0.14$) was observed for the left side. Finally, no association was observed between thalamic GABA+/Glx ratios and ETRS.

Figure 4.5. Correlation analyses. Correlation between the cerebellar GABA+/Glx ratio and the ETRS score for the voxel placements in the left (A) and right (B) hemisphere. The Spearman correlation coefficient (r) is reported in each plot. (Reproduced from Tapper S, Göransson N, Tisell A, et al. Essential Tremor: Cerebellar GABA+/Glx Ratio is Correlated with Tremor Severity. In manuscript.)

4.4 Technical Challenges in the Clinical Setting

There were several technical challenges during the acquisitions when performing the MEGA-PRESS measurements in the clinical setting.

4.4.1 fMRI Prior to MRS MEGA-PRESS

In the narcolepsy study, the fMRI was performed prior to the MRS MEGA-PRESS measurement. Several datasets were discarded, not only due to movements but also due to a large frequency caused by heating from the fMRI measurement. Figure 4.6 shows an example of the frequency drift when performing MEGA-PRESS measurements prior to and after a longer fMRI measurement. The system stability during the subsequent MEGA-PRESS measurement was severely affected.

Figure 4.6. Frequency drift. Illustration of the drift in synthesizer frequency between subsequent MEGA-PRESS measurements performed using a phantom prior to and after an hour of fMRI measurements. Apparent by this figure, the fMRI measurement causes heating of the MR system, which in turn generates frequency drifts, and thus, the stability of the subsequent MEGA-PRESS measurements was severely affected.

4.4.2 “Identical” MR Systems and Shimming

The data collection in the IBS study was affected by the change of MR system in the middle of the study. As evident in Figure 4.7, the stability of the newer but “identical” system was worse than for the older system. Thus, a larger spread in the resulting FWHM calculated by LCModel was observed for MR5, which in turn resulted in more discarded datasets. Furthermore, Figure 4.8 illustrates two examples of discarded datasets. As seen in these examples, the shimming of the voxel was insufficient, and as a consequence, the creatine and choline signals were inseparable or the spectrum was contaminated by lipid signals.

Figure 4.7. MR3 vs MR5. Comparison between MR3 (purple) and MR5 (green) looking at the spectral quality in terms of FWHM computed using LCModel. All data points originates from the IBS study, which was affected by the change of MR system.

Figure 4.8. Effect of a bad shim. Illustration of the consequences a bad shim of the voxel have on the resulting OFF spectrum. In the left spectrum, the creatine and choline signals are inseparable. In the right spectrum, a large lipid contamination is visible.

4.4.3 Movements during MRS

Figure 4.9 illustrates the effect on one dynamic affected by head movements during the MRS experiment. The movements resulted in a combination of phase errors, frequency shifts, and magnitude errors, which resulted in a reduction of the intensity of the resonances, where the amount of the reduction depended on the duration of the movements.

Figure 4.9. *The effects on an OFF-dynamic due to motion-induced artifacts. (B) Spectrum computed from a single dynamic acquired from a measurement performed without any intentional movements. (C) Spectrum computed from a single dynamic with intentional subject movements performed during the acquisition. (Reproduced from Tapper S, Tisell A, Lundberg P. (2017) How does motion affect GABA-measurements? Order statistic filtering compared to conventional analysis of MEGA-PRESS MRS. PLoS ONE 12(5): e0177795.)*

4.5 Increased Reliability in MRS Concentrations Using OSF?

No significant differences were observed in any of the resulting metabolite concentrations when analyzing the reference measurements using OSF and standard averaging (Figure 4.10 A). When the reference measurement was compared to the corresponding movement measurement, the resulting confidence intervals that represented the difference in concentrations were much larger (Figure 4.10 B). In addition, the estimated concentrations were generally lower when motion-induced artifacts influenced the spectra. Furthermore, for all three post-processing schemes, the more intense metabolites tCr and tNA were significantly different between the reference and the movement measurements. An improvement in these concentration estimates was observed when using the OSF technique; however, these concentrations were still significantly lower than those computed using the reference measurement. Moreover, the RAW PC scheme resulted in a slightly smaller difference in estimated concentrations than the RAW Dyn scheme. Finally, no improvements were observed for GABA+ or Glx when using OSF when artifacts were present in the spectra.

Figure 4.10. *The confidence intervals computed from the paired t-tests performed for the GABA+, tCr, tNA and Glx concentrations. (A) Paired t-tests performed between the three different post-processing techniques applied only to the reference measurements. (B) Paired t-tests performed between the measurements with movements and the reference measurements analyzed using the same post-processing method. (Reproduced from Tapper S, Tisell A, Lundberg P. (2017) How does motion affect GABA-measurements? Order statistic filtering compared to conventional analysis of MEGA-PRESS MRS. PLoS ONE 12(5): e0177795.)*

4.6 Artifact Detection and Elimination Using JKC

4.6.1 *Delayed Effect of the Movements*

As shown in Figure 4.11, the first spectrum in the movement episode (M1) was unaffected by the head movements. Brief delays due to reaction times were unavoidable since the movements were initiated by vocal commands. However, these reaction times were not long enough to also affect the excitation of the first spectrum after each movement episode (AM1). In addition, regardless of which window was used, higher computed correlation coefficients were observed for the M1 spectra; meanwhile, generally lower correlation coefficients were observed for the AM1 spectra. Therefore, the actual movement paradigm used for the computation of the sensitivities and specificities was shifted one excitation in time.

4.6.2 *Optimal Methodology Using Training Data*

The resulting correlation coefficients were much larger using the water window compared to the metabolite window. In addition, when using the metabolite window, a notable difference was also observed between the ON and OFF edited data, which was expected due to the SNR loss in the 1.0-4.0 ppm range as a result of the ON editing. Furthermore, the optimal cutoffs for the correlation coefficients were computed using the Youden index (OFF: 0.7618 versus 0.9879; ON: 0.7451 versus 0.9905), comparing the metabolite versus water windows. In addition, using the water window, both higher sensitivity (OFF: 0.5455 versus 0.7190; ON: 0.7308 versus 0.7846) and higher specificity (OFF: 0.9750 versus 0.9828; ON: 0.7480 versus 0.9605) were observed than when using the metabolite window. In conclusion, the water window was more useful for detecting the movement artifacts, and was therefore only used in the subsequent analyses.

4.6.3 *Artifact Detection Using JKC*

The optimal methodology (water window, cutoffs: 0.9879 [OFF], 0.9905 [ON]) was applied to the validation data, which generated the artifact detection shown in Figure 4.12. A good agreement between the movement paradigm and the detected artifacts was observed, and both high sensitivities (datasets 2: 0.7091 [OFF] and 0.7143 [ON]; datasets 3: 0.7364 [OFF] and 0.7541 [ON]) and high specificities (datasets 2: 0.9663 [OFF] and 0.9536 [ON]; datasets 3: 0.9430 [OFF] and 0.9221 [ON]) were obtained. Furthermore, for one volunteer (volunteer 10) specifically, there were two longer patches (blue label) that were characterized as artifacts, but free from artifacts according to the movement paradigm, which may indicate that a shift in the voxel position occurred.

Figure 4.11. Movement paradigm in context of the pulse sequence and correlation coefficients obtained using the 2 windows. (A) The pulse sequence consists of a water suppression (WS, 212 ms), an excitation (Exc, 68 ms), a readout (512 ms), and a delay (TR = 2000 ms). The vocal instruction for the start of the movement episode was given when the excitation was performed, which was indicated by a noise from the scanner. Thus, there were 2 reaction times: 1 reaction time when giving the instruction, and 1 reaction time for the volunteer to obtain the instruction and start the movement. The same reaction times also applied to the communication about stopping the movement. (B) Correlation coefficients were computed using the metabolite window for the acquisitions containing movement (M1-M8) and the 3 following excitations after an episode (AM1- AM3). Each value corresponds to a black dot, and the mean correlation coefficient is indicated by the blue dot. (C) The same as in (B) but computed using the water window. (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med.* 2018;00:1-15)

Figure 4.12. Artifact detection using the water window applied to validation datasets 2 (A) and 3 (B). Each individual measurement consisted of $M = 20$ OFF and $M = 20$ ON interleaved dynamics, in which each dynamic consisted of 8 phase cycle steps. In this figure, every measurement from each of the 12 volunteers (V) is depicted as an $M \times 16$ matrix corresponding to all excitations. Thus, each “pixel” in the figure corresponds to 1 excitation and was color-coded according to the result of the filtering. Black, no movement detected according to the paradigm (true negative [TN]); red, no movement detected but movement according to the paradigm (false negative [FN]); green, movement detected according to the paradigm (true positive [TP]); blue, movement detected but no movement according to the paradigm (false positive [FP]). (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med.* 2018;00:1-15)

4.6.4 JKC Spectra after Elimination of Detected Artifacts

Using JKC, the detected spectra were eliminated prior to averaging, and compared with the results from standard averaging without any data elimination (Figure 4.13 and Figure 4.14). Only small visible differences were observed between the resulting mean spectra using the two methodologies. However, when the 36 individual spectral residuals were investigated, the differences were more obvious, especially, for the movement measurements when comparing JKC and standard averaging (Figure 4.13 B and Figure 4.14 B). Meanwhile, no large differences were observed between JKC and standard averaging when the reference data were used. Furthermore, for the difference spectra (Figure 4.14 B column 2), the residual appeared to be both positive and negative, in close proximity to 3 ppm, where the GABA signal was detected. Finally, when the movement and reference measurements obtained from the same protocol were compared, it was apparent that the resulting residuals were both positive and negative, although more of the residuals were positive (Figure 4.13 B and Figure 4.14 B, columns 3-4).

Figure 4.13. OFF spectra and residuals obtained using the 2 different methods applied to the reference and the movement measurements. (A) Two mean spectra (over 36 spectra), 1 in black and 1 in red, and the small plot above each spectrum show the residual between these 2 mean spectra. (B) Residuals from the individual measurements (total of 36 in each plot). The asterisks in columns 3 and 4 in (B) highlight 2 reference measurements with less SNR than the corresponding movement measurement. (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med.* 2018;00:1-15)

Figure 4.14. The GABA+ spectra and residuals obtained using the 2 different methods applied to the reference and the movement measurements. (A) Two mean spectra (over 36 spectra), 1 in black and 1 in red, and the small plot above each spectrum show the residual between these 2 mean spectra. (B) Residuals from the individual measurements (total of 36 in each plot). The asterisks in columns 3 and 4 in (B) highlight 2 reference measurements with less SNR than the corresponding movement measurement. (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med*. 2018;00:1-15)

4.6.5 Increased Reliability in MRS Concentrations Using JKC?

The JKC and standard methods were compared when applied to the reference measurements, which resulted in relatively small and not significantly different confidence intervals (Figure 4.15, green label). In general, when the movement and reference measurements were compared using the standard (Figure 4.15, red label) and JKC methods (Figure 4.15, black label), a larger difference was observed for standard averaging than for JKC. Furthermore, there was a significant difference in the computed concentrations (except for Glx) between the movement measurement and the reference when standard averaging was used. Those significant differences were no longer detectable for the tCho, tNA, and GABA+ concentrations when the implemented JKC method was used. Nevertheless, the p-values were very close to the significance limit for every metabolite except tCho, which was also illustrated by the confidence intervals.

Figure 4.15. 95% confidence intervals. The 95% confidence intervals obtained when performing the paired t tests. Green, JKC versus standard averaging applied to the reference measurement; red, reference versus movement measurements using the standard technique; black, reference versus movement measurements using the implemented JKC method. These confidence intervals were computed for the total choline (tCho), total creatine (tCr), tNA, Glx, and GABA+ concentrations. The mean concentration differences are indicated by the white lines. The corresponding P values are also illustrated in the figure, and color-coded according to the corresponding interval. (Reproduced from Tapper S, Tisell A, Helms G, Lundberg P (2018). Retrospective Artifact Elimination in MEGA-PRESS Using a Correlation Approach. *Magn Reson Med.* 2018;00:1-15)

4.7 JKC vs OSF vs Standard Averaging

Figure 4.16 shows the confidence intervals when comparing the reference to the movement measurement using the standard averaging method, the implemented JKC method, and the OSF method using the RAW PC scheme. Using solely the OFF data, the JKC method performed best for our set of MRS MEGA-PRESS data, which was especially evident in the tCho and tCr concentrations. Furthermore, for the tNA concentrations, equal improvements were observed using both JKC and OSF. Finally, when investigating GABA+ concentrations, only marginal differences were observed between the different methods; however, the reference and movement GABA+ measurements were no longer significantly different when JKC or OSF was used.

Figure 4.16. 95% confidence intervals. The 95% confidence intervals obtained when performing paired *t*-tests, when comparing the reference versus movement measurements using the standard technique (red), the implemented JKC method (black), and the OSF technique with the RAW PC scheme (blue).

4.8 MRSI Measurements with Spiral Readout

4.8.1 Localization Using Two Different Coils

Figure 4.17 shows representative spectra from a 1 mL voxel in the Braino-phantom when using different localization techniques, enabled/disabled MEGA-editing, and the two different head coils. The Cho, Cr, and NAA resonances were clearly visible in all the four unedited spectra, and also, some Glx signals were fitted. The MEGA-edited difference spectra show the expected negative NAA signal, although the spectra were very affected by noise. Furthermore, the PRESS spectra had approximately the same SNR level as the sLASER spectra; meanwhile, the spectra collected using the T/R coil had a higher SNR level than the spectra collected using the 12-channel head coil.

Figure 4.17. Braino-phantom measurements using different volume selection and spectral editing. Representative spectra corresponding to the dark red box in A-D (Braino phantom), using PRESS (E-H) and sLASER localization (I-L) without MEGA-editing (E-F, I-J), and with MEGA-editing (G-H, K-L). The first and third column show representative spectra when using the 12-channel head coil, meanwhile the second and fourth column show representative spectra when using the T/R-head coil. The resonances in the spectra are assigned in (E, G). (Reproduced from Tapper et al, 2019, *nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges*, in manuscript.)

Figure 4.18 illustrates the corresponding concentration maps. The different shapes of the maps were highly dependent on the excitation profile of the coil, and also on the localization technique used. Furthermore, as expected, the MEGA editing completely removed the creatine signal from the spectra.

Figure 4.18. NAA and creatine in the Braino phantom. Resulting NAA (upper row) and Cr (lower row) maps computed for the Braino measurements when using the 12-channel head coil (A-E) and T/R- head coil (F-J). The VOIs ($100 \times 100 \text{ mm}^2$) were placed in the center of Braino (A,F). The maps were computed for the measurements with PRESS and sLASER localization, without MEGA- editing (B-C, G-H) and with MEGA-editing (D-E, I-J), respectively. The creatine signal at 3.0 ppm is removed when using MEGA-editing. The white line indicates from which row the intensity profile above each map originate from. Each NAA-map was normalized, and corresponding Cr- map was scaled using the same value as in the normalization of the NAA-map.

4.8.2 GABA Detection Using MEGA-Edited MRSI

Two different VOI sizes were used when investigating both PRESS and sLASER localization in combination with MEGA editing. As shown by Figure 4.19, the fitted GABA signal was clearly visible when both localization techniques and VOI sizes were used. However, due to the short acquisition time (1 min 45 s), the resulting spectra had a low SNR, which was apparent in the residuals.

Figure 4.19. Cubic GABA + Creatine Phantom. Representative spectra originating from the dark red box in A ($\text{VOI} = 100 \times 100 \text{ mm}^2$) and D ($\text{VOI} = 200 \times 200 \text{ mm}^2$) using MEGA-PRESS (B and E) and MEGA- sLASER (C and F). There were no larger differences in the resulting representative spectra for the two different VOI sizes. However, due to the short acquisition time and the 1 mL voxel size, the SNR in the representative spectra are limited. (Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.)

4.8.2.1 Extended MEGA-sLASER Measurements

As shown by Figure 4.20, the conventional 27 mL SVS MEGA-PRESS measurement (10 min 40 s) clearly showed the GABA resonances in the difference spectrum. This was also the case in the difference spectra originating from one 1 mL voxel in the long MEGA-sLASER MRSI measurement (17 min). However, a loss of SNR was observed, as expected, in addition to some artifacts from eddy currents that were visible in the spectrum. Moreover, from the resulting difference spectrum computed for Braino, it was possible to identify co-edited Glu present in the spectrum appearing at 3.74 (^1CH) and 2.34 ppm ($^3\text{CH}_2$)(and at c. 2.08 ppm for $^2\text{CH}_2$).

Figure 4.20. Extended MEGA-editing measurements in GABA + Creatine phantom and the spherical metabolite phantom. VOI placement and representative spectra. (A) Results from a conventional single-voxel MEGA-PRESS (27 mL, 30 x 30 x 30 mm³). (B) Representative resulting difference spectrum revealing the GABA signal that originates from the 1 mL voxel highlighted in dark red. (C) The representative resulting difference spectrum originating from the 1 mL voxel highlighted in dark red. Since Braino does not contain any GABA, the difference spectra only shows co-edited glutamate signal and NAA. (Reproduced from Tapper et al, 2019, nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges, in manuscript.)

4.8.3 Varying Maximal B_1 Amplitude

Only minor differences were observed between the resulting H_2O maps when the maximal B_1 amplitude was decreased in increments of $1 \mu T$ from $20 \mu T$ down to $13 \mu T$ (Figure 4.21). This result implies that the adiabatic condition was fulfilled to the same extent for all B_1 amplitudes between $13 - 20 \mu T$.

Figure 4.21. Varying B_1 -amplitude. Resulting H_2O map computed using a Max B_1 of $20 \mu T$ (A) with a $1 \mu T$ decrease in each measurement (C-E, G-I) down to a Max B_1 of $13 \mu T$ (J). The $100 \times 100 \text{ mm}^2$ VOI (A, F) was placed in the center of the phantom. The single channel T/R-coil was used in these measurements. (Reproduced from Tapper et al, 2019, *nD Quantitative Chemical Shift Imaging of GABA in the Human Brain: Advantages and Challenges*, in manuscript.)

4.8.4 Drifts in K_0

Only minor differences were observed in the drifts in K_0 between the eight different angular and temporal interleaves. This drift was measured for a range of different datasets and conditions, and the magnitude of the drift in K_0 in the different datasets was similar. Due to the slight drift between the temporal interleaves, the resulting FID from one voxel in the MRSI grid appeared to consist of two different FIDs, each corresponding to every other sample. This effect was apparent in the corresponding spectra by artifacts appearing at the edges of the spectra. Finally, in contrast to the temporal interleaves, the effect of drift between angular interleaves was spread out across the resulting spectra.

5. DISCUSSION

5.1 Application of MEGA-PRESS on Narcolepsy

The main finding of this study was that the narcolepsy patients were characterized by increased deactivation of the DMN, where the deactivation was positively correlated with activity within task-related frontal regions. This task-related increased deactivation of the DMN has previously been shown to be linked to mental effort [90, 91], which may suggest that narcolepsy patients either find any task effortful or their cognitive resource allocation system is overly focused on maintaining adequate levels of attention. Furthermore, we also observed that an increased deactivation in mPFC during the encoding of sentences was associated on a trend-level with decreased GABA+ and increased Glu concentrations in narcolepsy; meanwhile, the opposite pattern was observed for the healthy controls. Moreover, a task-related increased level of deactivation in DMN has previously been observed to correlate with increased GABA concentrations in healthy controls [74, 75]. In addition, since the opposite relationship was observed in narcolepsy, this points toward an active suppression or some form of metabolic dysregulation of at least the anterior portion of DMN. However, it is important to note that the relationships between GABA+ and Glu and deactivation in mPFC during the task were only at a trend level. Finally, taken together, the fMRI and MRS results may suggest an imbalance in the allocation of cognitive resources in narcolepsy in favor of maintaining sustained attention during the task.

5.2 Application of MEGA-PRESS on IBS

We observed increased mPFC GABA+ levels in women diagnosed with IBS comorbid with a high severity of anxiety symptoms. In addition, a reduced functional connectivity between mPFC and adjacent ACC was observed for these women, which may suggest a disruption of both neurotransmission and functional connectivity within the prefrontal regulatory circuits, which in turn may be involved in the commonly increased severity of anxiety and depression symptoms often observed in IBS. Consistent with our findings, studies have previously shown relationships between prefrontal GABA, functional coupling within corticolimbic circuits, and anxiety in healthy volunteers [29, 30]. Furthermore, our findings were also consistent with previous reports of abnormal top-down modulation in IBS and other disorders of abnormal brain-gut communication on both a functional and biochemical level [92, 93]. In addition, we did not find any associations between mPFC GABA+ or Glx and disease-

related measures such as symptom severity or pain. Also, only distinct differences in mPFC GABA+ were detected between the subgroups based on anxiety symptom severity, which further highlights the heterogeneity of IBS [94].

5.3 Application of MEGA-PRESS on ET

In this study, we found a positive correlation between cerebellar GABA+/Glx ratios and tremor severity in ET patients scheduled for DBS surgery. This positive correlation was mainly driven by a negative correlation with Glx (or Glu, since Glx mainly consists of Glu), which may suggest a disturbance in cerebellar excitatory neurotransmission. Recently, much research on ET has been focused on investigating the cerebellum, and in particular the Purkinje cells and the dentate nucleus [95, 96], where several morphological aberrations such as Purkinje cell reduction have been identified. However, simultaneously, a number of studies have failed to find any evidence of this Purkinje cell loss [97-99]. As previously described, the Purkinje cells receive direct excitatory inputs from climbing fibers and parallel fibers [12] (Figure 5.1). Subsequently, a density loss of climbing fiber-Purkinje cells would result in a lower degree of inhibition of the dentate nucleus. This reduced inhibition of the dentate nucleus may in turn lead to an imbalance of the cerebellar error correction, based on a relative decrease of the effect induced by the excitatory feedback response from the sensory system of ongoing movement. Our result was consistent with previous reports of a disturbance both in Purkinje cells and at the dentate nucleus in ET patients. Our interpretation was that the observed GABA+/Glx ratios could potentially suggest an altered excitatory neurotransmission in ET.

Furthermore, no morphological changes have previously been found for other regions involved in the motor network such as the thalamus. These previous reports agree with our results since we did not observe any associations between thalamic GABA+ or Glx concentrations and ETRS. However, Barbagallo *et al.* have shown increased thalamic Glx in ET compared to healthy controls, and also that Glx was associated with tremor severity [100]. However, these contradictory results may be due to different methodological approaches in the MRS quantification. Finally, it was important to note that our sample size was rather small, which may be the cause of the non-significant results.

Figure 5.1. Pathways of cerebellar error correction in essential tremor. The suggested neuro-degeneration results in a relative decrease of excitatory neurotransmitter Glu, resulting in a hypothetical net decreased inhibitory effect from Purkinje cells on the dentate nucleus in ET. This will in turn lead to an imbalance in the cerebellar error correction, based on a relative decrease of the effect induced by the excitatory feedback response from the sensory system of ongoing movement. The MEGA-PRESS voxels were acquired at 3 T, separately for R and L cerebellum, including both Purkinje layer, and the subcortical dentate nucleus. The MEGA-PRESS-voxel (either R or L) is represented by a dotted square. Labels (1) and (2) represents two specifically reported locations of structural-functional aberrations in ET versus healthy controls. Excitatory and inhibitory synaptic stimulation is represented by green and yellow circles, respectively. (Image of a Purkinje cell was obtained from Gray's anatomy, 1918 edition, in the public domain). (Reproduced from Tapper S, Göransson N, Tisell A, et al. Essential Tremor: Cerebellar GABA+/Glx Ratio is Correlated with Tremor Severity. In manuscript.)

5.4 Limitations in SVS MEGA-PRESS in the Clinical Setting

We experienced some limiting aspects using SVS MEGA-PRESS in the clinical setting, which are described below.

5.4.1 Large Voxel

A large voxel (20 - 30 mL) is needed in the MEGA-PRESS experiment in order to obtain a sufficiently high SNR in the spectrum to reliably quantify the low-SNR GABA signal. However, there are other limiting aspects to this approach, especially in the clinical research applications. For example, in the IBS study, a smaller voxel was desired in order to measure GABA within the specific sub-regions of the prefrontal cortex. Also, in the ET study, there were negative aspects to the larger voxel used. Our voxel included part of the cerebellar cortex, in combination with the deep cerebellar nuclei (*e.g.* dentate nucleus) and white matter tracts in the cerebellum. Due to this limited spatial resolution, it was difficult to determine whether ET was characterized by morphological abnormalities in specific cerebellar regions, or whether the biochemical alterations were limited to certain parts.

5.4.2 Long Acquisition Time - Movements

The acquisition time is another important factor that is directly related to the resulting SNR in the spectra. During the SVS MEGA-PRESS measurements, we collected 40 dynamic averages which corresponded to an acquisition time of 10:40 min. During this long acquisition time there is a greater risk of the participant moving during the measurement. The consequences of the movements have been shown to reduce the concentration estimates, and also, there is a greater risk of a shift in the voxel placement occurring during the acquisition. In addition, scanner time is precious, and when practicing clinical research, there may not be time for several measurements to be performed if they are above 10 min. Also, since we only measure one region at a time, there may be a time constraint in how many regions that can be investigated in a research application due to this longer acquisition time.

5.4.3 MRS in Combination with Other Techniques

When performing clinical research applications, studies often aim to investigate more than just MRS measurements. For instance, we also performed fMRI in the studies investigating both the narcolepsy and IBS patients. The narcolepsy study was our first experience of using MEGA-PRESS at our site, where we chose the protocol design of performing MEGA-PRESS directly after the fMRI measurement. Although it is interesting to see the effect on the MRS concentrations after the fMRI stimuli, the consequence of a large frequency drift severely impacts the spectra negatively [101]. As a consequence, we observed a large spread in the computed GABA+ and Glu concentrations, which could

explain the lack of significance in the associations between activation and neurotransmitter levels in mPFC in narcolepsy.

In addition, since the fMRI measurements in the narcolepsy study were performed in combination with simultaneous EEG, the participants still had the EEG cap on during the MRS measurements (but not activated). Moreover, as was evident from the fMRI measurements, subject movements were present during the data collection, which may be explained by this EEG equipment, which the participants may considered uncomfortable. Also, it was not clear how much the EEG cap affected the MRS measurement in terms of inducing field inhomogeneities.

Currently, there are no DBS stimulators conditional for 3 T; thus, in the ET study, it was not possible to perform repeated measurements of GABA at 3 T post DBS surgery. However, this may be possible in a few years, when a sufficiently acceptable set of MR safety conditions have been developed.

5.4.4 Identical MR Systems?

In the IBS study we observed that two identical MR systems may perform differently when using the same protocol parameters, settings and routine. Using the new system (MR5), we noted that the overall quality of the data collected with the voxel placed in the mPFC began to decline. Since it is relatively well known that it is difficult to achieve a good shim in the prefrontal cortex, we investigated the FWHM obtained from LCModel as our quality parameter. We observed a significantly worse overall shim using the new system, which highlights that one cannot expect that identical systems will perform in an identical manner.

5.4.5 Chemical Shift Displacement Error

As previously described, the CSDE is a large problem particularly when using a PRESS family pulse sequence at high field strengths. The effects from this error was not addressed in our studies. A possible solution to this problem is to replace the PRESS localization with sLASER or LASER localization, which due to the properties of the adiabatic selection would minimize this error.

5.4.6 Frequency Drift

A large frequency drift was present in some of the clinical datasets, which appeared to be directly connected to the protocol performed prior to our measurement. As described before, this frequency drift was also present in all datasets in the narcolepsy study, since the fMRI measurement was performed prior to MRS. When a larger drift is present during the measurement, there is a risk that the editing pulses will miss their target frequency, and thus a risk of insufficient editing. Also, if the frequency correction performed in the post-processing step is insufficient this may result in subtraction artifacts due to misalignment between OFF and ON spectra. However, most of the data were

without a larger frequency drift (< 3 Hz), and frequency correction based on the water residual did not appear to yield any large subtraction artifacts in the resulting difference spectra. Another approach is to use spectral registration, which is a method for phase and frequency correction that is not based on any specific resonance [69].

5.4.7 GABA+ or GABA?

During the MEGA editing, we placed our pulses symmetrically around the water signal at 4.7 ppm, and as a consequence, we measured GABA+. This measure of GABA+ included a fraction of macromolecules, and probably also homocarnosine, which is often considered as a constant fraction (about 40%), and thus a change in GABA+ mainly reflects changes in GABA. In addition, there are options of removing these co-edited macromolecular signals, *e.g.* by placing the editing pulses symmetrically around the macromolecules at 1.7 ppm [62, 102]. However, from our own experience, and also as previously reported, this results in a signal loss of GABA in the spectrum, which complicates the quantification.

5.4.8 Glutamate Quantification Using MEGA-PRESS

Often when measuring GABA, we are also interested in measuring glutamate, due to the inhibitory and excitatory relationship between these two metabolites. The MEGA-PRESS experiment was optimized for GABA detection and not ideal for separating glutamate from glutamine. However, as described previously, due to time constraints it may not be possible to perform a separate measurement with the glutamate as a target. Furthermore, one study showed good agreement between unedited Glx quantification using PRESS at 1.5 T, and quantifying Glx using solely the OFF-edited spectra [103]. In the narcolepsy study, we investigated the Glu concentrations from the OFF spectra, which may not have been optimal since we cannot guarantee that the Glu estimate was fully separated from Gln. Therefore, in the subsequent studies (IBS and ET), we instead focused on the Glx measurement.

5.4.9 Absolute Quantification

We did not perform any segmentation of the voxel in order to find the tissue composition of WM, GM, and CSF, within the voxel, which is a limitation of this methodological approach. However, many of the effects are removed when concentration ratios are quantified. We used a separate water reference acquired within the same voxel to scale our concentration estimates in order to try to achieve absolute units of milli-molar. However, there are many corrections that need to be performed correctly in order to obtain absolute concentrations. In addition, our approach is especially limiting when the tissue composition within the voxel has changed between a patient population and healthy controls, which can be caused, for example, by atrophy or edema. In this case, it is hard

to interpret whether this resulting change in concentration between two groups was caused by an altered neurotransmission or in a change of tissue composition within the voxel.

5.4.10 Quality Measurements

When LCModel is used in the quantification, the resulting fit is accompanied by the parameters FWHM, SNR, and %SD, which describe the quality of the data and fit. Unfortunately, these parameters only differed marginally between most of the datasets. For example, the %SD typically were 2 or 3%, which in this case would indicate that the best model fit (in terms of SD expressed in mM) was obtained for the lowest concentration. In addition, the FWHM estimate was reported in incremental values resulting in a crude measure of the linewidth. Also the SNR measurement could be misleading sometimes, for instance when lipids contaminated the spectra (*e.g.* due to CSDE) and therefore generated a very high SNR. Therefore, a reliable quality measurement or constant visual inspection of output spectra is necessary in order to trust the resulting concentrations.

5.5 Retrospective Approaches for Quantification Improvement

Retrospective approaches for data improvement were investigated in order to increase the reliability of the resulting concentrations by removing artifacts from the datasets. Moreover, a retrospective approach was chosen since prospective techniques such as navigators or optical tracking systems are often difficult to implement, expensive or time-consuming.

5.5.1 Inducing Artifacts in the MRS Data

As previously described, we used a movement paradigm in order to induce artifacts in a “controlled” manner in our MRS data. Of course there are limitations to this approach, for instance regarding finding the correct voxel placement after a movement episode or guaranteeing that spectra actually were without artifacts when supposed to be according to the movement paradigm. In addition, since motion correction was not the only purpose, and the movement artifacts appeared to result in a systematic reduction of signal in the spectra due, for example, to phase errors and frequency shifts, this may have affected the overall performance of the chosen retrospective approach.

During the correlation analyses, a one-step time lag was observed in the spectral effect from the movement episodes compared with the intended movement paradigm. This time lag cannot be explained by the expected reaction times for the volunteers to start and stop the movement. Therefore, one possible explanation for this phenomenon was a T_1 relaxation effect, which meant that a steady state was not obtained in the excitation directly after the movement stopped.

5.5.2 Order Statistic Filtering

The OSF technique could be used for all datasets regardless of whether or not artifacts were present in the data. As previously described, a general reduction of the estimated concentrations was observed when movement artifacts influenced the spectra. Moreover, since the OSF technique consists of a rank-ordering followed by the usage of the median filter, a systematic reduction of signal intensity would also result in an underestimation of the median. Even though the median spectrum might be misleading here, theoretically, it was still an improvement compared to using the mean spectrum.

The initial hypothesis was that the RAW PC scheme was the most effective procedure due to the elimination of motion artifacts prior to the combination of phase cycle steps. Therefore, the slightly better performance of the RAW PC scheme compared to the RAW Dyn scheme, could be explained by the earlier removal of the artifacts.

Only a trend-level improvement was observed in the resulting concentrations for the most intense resonances tCr and tNA, while no such improvement was observed for the low-SNR and scalar coupled metabolites GABA+ and Glx. There may be several explanations for this lack of improvement in the GABA+ concentrations computed using OSF. First, the GABA+ quantification was completely different to the quantification of the other metabolites (difference spectra vs OFF spectra). Second, the GABA+ signal was characterized by a considerably lower SNR than the creatine or NAA singlets, and the SNR of GABA may not be high enough for OSF to be useful. Third, as a consequence of these movements, LCModel may also have difficulties fitting spectra with lower SNR reliably. Finally, as previously discussed, this methodological approach was not optimal for Glx quantification, and from previous experiences, when the SNR is reduced in the spectra, LCModel has difficulties, especially fitting the Glx signal, which may explain the high uncertainty of the resulting Glx concentrations in this study.

5.5.3 Implemented JKC Method

The implemented JKC method characterized the majority of the artifact-influenced spectra in the validation datasets correctly. After elimination of the detected artifact-influenced spectra, the computed concentrations were closer to those obtained from the reference datasets. Using the reference datasets, JKC and standard averaging performed equally, which suggests that JKC can be used independently, whether or not the datasets have been contaminated by motion artifacts.

The water window was found to be superior to the metabolite window in detecting instructed motion, which was expected due to the higher SNR of the water residual (weak water suppression) than of the metabolite signals in the analysis window. Furthermore, using the water window, the correlation

coefficient cutoffs were 0.9905 for ON data and 0.9879 for OFF data. This large number of decimals in the resulting cutoffs was required for optimal detection, as there would have been a large stepwise difference in the results if a cutoff limit of, for example 0.98 or 0.99 had been chosen. Furthermore, some undetected spectra were observed at the beginning and end of some movement episodes as a result of the trade-off between obtaining a maximized sensitivity and specificity. Finally, these cutoffs were calibrated for our type of data, and clearly cannot be used as general cutoffs. For example, the cutoffs could either be recalibrated for other data types or adjusted manually. Also, another approach may be to eliminate a predefined number of spectra with the lowest resulting correlation coefficients.

As shown by Figure 4.12, there were two longer blue sections in the results for volunteer 10, which according to the movement paradigm were spectra that were incorrectly characterized as being artifact-contaminated. This result suggests that the original voxel placement was not regained after the movement episodes, and thus, the spectral appearance was altered, which in turn explains the loss of correlation between the spectral windows. Furthermore, if many spectra were characterized as artifact-contaminated, this would be a strong indication of poor general quality of the dataset. Therefore, the application of JKC may be a suitable technique for quality control of an MRS dataset and may indicate if a shift of the voxel occurred during the measurement.

5.5.4 OSF or JKC or Standard Averaging?

As shown by Figure 4.16, both OSF and JKC reduced the concentration differences between the reference and movement measurements obtained from the same protocol, where the largest improvements were observed for tCho, tCr, and tNA. Moreover, focusing on the tCho and tCr concentrations, JKC clearly performed better than both OSF and standard averaging. This result might be explained by the usage of the water window, which focused on a high correlation in close proximity to the tCho and tCr concentrations. In this case, the GABA signal should also be included in this reasoning, and the explanation here may instead be in the subtraction or low SNR of GABA. Finally, note also that the OSF concentrations were re-calculated for the purpose of this comparison, and differences in the quantification apply.

To summarize, using standard averaging is not recommended when artifacts influence the data, and both OSF and JKC appear to increase the reliability of the resulting concentrations, although JKC seems to perform better for our type of data and artifacts.

5.5.5 Limitations

We did not use any separate hardware monitoring of the movements since this is not widely available and may be technologically challenging to use. As is evident in Figure 4.13 and Figure 4.14, the reference measurements were not

guaranteed to be without artifacts, and there were two reference datasets worse in quality than several of the movement measurements. After a closer inspection of these datasets, it was concluded that the volunteer had difficulties remaining motionless during the acquisitions. Furthermore, this observation strongly highlights the importance of a retrospective method for MRS quality control and artifact detection, since artifacts and poor quality data can influence the MRS data also when examining healthy controls.

In both retrospective approaches, it was necessary to use raw data in order to obtain the spectra from each excitation. Although this type of data requires slightly more post-processing, these extra steps could be incorporated within the conventional post-processing sequence; however, requiring a few more seconds of processing time.

5.6 MRSI MEGA-sLASER Measurements

MRSI with full brain coverage in combination with a minimal voxel size for the purpose of GABA detection would be extremely useful in clinical research applications where disorders such as narcolepsy, irritable bowel syndrome, or essential tremor are investigated. Using our fast MEGA-sLASER MRSI sequence, we could detect GABA within a short time-frame using a grid resolution of 1mL. Since the main aim was to summarize an initial experience with this sequence on a conventional clinical scanner using phantom measurements, the technical challenges we explored were significant. However, many of these challenges are common issues when performing demanding applications.

5.6.1 Spiral Readout

Spiral was primarily chosen since it is very fast readout approach. In addition, since K_0 is oversampled, spiral also has the advantage of being rather insensitive to movements, which previously was shown to be important in this thesis. Prior to reconstruction, we also observed drifts in K_0 between the different angular and temporal interleaves. A line-broadening may reduce the effect between the different temporal interleaves; however, reducing the drift between angular interleaves after reconstruction is a challenge.

5.6.2 Localization Using Different Detection Coils

Comparing sLASER and PRESS, we observed a skewness of the resulting concentration maps when using PRESS, which was a result of either the excitation profile or insufficient refocusing. In addition, PRESS is also more sensitive to B_1 inhomogeneities, which may further explain these deviating map shapes. Furthermore, no difference in spectral SNR was observed between sLASER and PRESS, which indicated no loss of signal when using sLASER. Comparing the 12-channel and T/R head coils, apparent differences were

observed in the map shapes due to the number and position of the coil elements. As expected, more pronounced edges were observed using the 12-channel coil; meanwhile, a circular and more pronounced middle map was observed for the T/R coil (one channel). Furthermore, a higher SNR was observed when the T/R coil was used, which may be a consequence of the higher B_1 amplitude or alternatively due to the relatively enhanced signal originating from the center of the coil.

5.6.3 MEGA Editing

GABA was detectable from 1 mL voxels during a 1:45 min measurement; however, as expected, the SNR was not very high compared to the control experiment using a 27 mL voxel during a 10:40 min measurement. Even though several voxels are quantified at a time, a very long measurement (17 min) may not be realistic in the clinical setting due to movements or drifts. In addition, it is important to remember that our phantom included a high concentration of GABA, which was much higher than the physiological GABA concentration within the brain. Moreover, from the longer Braino measurement, it was possible to detect co-edited glutamate in the difference spectrum. Although, it is not recommended to quantify Glu or Glx from the difference spectrum, it would be valuable to also measure Glx from the same measurement due to the inhibitory/excitatory relationship between GABA and Glu.

5.6.4 Technical Challenges

5.6.4.1 MR System

The sequence was developed for a conventional clinical 3 T MR system with only a maximal available B_1 of 13.5 μT when transmitting with the quadrature body coil, which could be negatively compared with other scanners that can utilize twice as much maximal B_1 . Although our T/R head coil can achieve a maximal B_1 of 20 μT , this also results in an increased field inhomogeneity and decreased SNR. However, when changing the maximal B_1 throughout the acquisitions, this did not appear to have any effect on the resulting water maps, which may be explained by the fact that the adiabatic condition was fulfilled to the same extent when using a maximal B_1 between 13-20 μT .

Furthermore, the echo time (TE) is an important aspect when performing GABA quantification, and TE is closely related to the maximal B_1 . During the acquisitions, we had to use a TE of 80 ms in order to have time to perform both sufficient MEGA editing and adiabatic refocusing. As a consequence, we needed to reduce the available time for gradient spoiling, which in turn generated the spurious signals that we occasionally observed in the spectra. Thus, increasing B_1 would allow for a reduced TE, longer MEGA editing pulses, or more time for gradient spoiling.

5.6.4.2 Eddy Currents

LCModel performed the eddy current correction using the water reference. Although an improvement was observed in the corrected spectra, there were still some eddy current artifacts left in the spectra. Several studies have highlighted the importance of eddy current correction, especially when performing a spiral readout [104, 105]. Since the first approach of using Klose eddy current correction applied to the individual voxels did not perform adequately, LCModel was an alternative at this point. The next step approach would be to investigate possibilities for corrections prior to reconstruction of the spirals, not only for the purpose of eddy current correction but also for correction of the observed drifts between the spirals.

5.6.4.3 Using Black-Box Software

As both ReconFrame and LCModel are ‘black-box’ programs, where the user does not know the exact details behind the algorithms within the program, this could be seen as a limitation. However, optimal re-gridding and reconstruction of spirals or metabolite quantification are complicated procedures, which demands much refinement and experience. Although one may wish at times to have more control over the specific post-processing steps in the software, at this point, we chose to use these software packages for the reason mentioned above. In addition, a known scaling issue within ReconFrame has recently been revealed, which currently is being investigated.

5.6.4.4 Memory Issues

During the acquisitions, we experienced several memory overflow related crashes of the scanner host due to limited storage space on the disk for the raw data, which highlights the importance of keeping an eye on the disk space during the measurements. In addition, using the 12-channel coil when acquiring more than eight averages of the 2D data, we could not reconstruct the spirals in ReconFrame due to memory issues. These memory issues in combination with the difficulties handling huge datasets, were the reasons we did not go beyond 2D measurements.

5.6.4.5 Philips Scanner Updates

The frequent scanner updates from Philips has been another limiting factor that has been very time-consuming in this project. For every update, a new patch needs to be implemented, as well as corresponding new versions of Pack ‘n Go, ReconFrame and other scripts, which in turn need iterations of testing even before working at the same level as the patch for the previous update.

5.7 Future Work

First of all, and as usual, all three clinical applications need further investigation and follow up studies in order to confirm our results and to draw reliable conclusions about the disorders in question. Furthermore, MR systems are continuously improving and new techniques for acquisition, post-processing and quantification are continuously emerging, and therefore, one can always improve the metabolite quantification. In our case, several improvements could be implemented in the absolute quantification of GABA in terms of for example voxel segmentation and more appropriate basis sets.

In addition, as shown by the retrospective methods investigated in this thesis, it is important to have procedures for quality control and improvement of datasets that are affected for instance by movements or other artifacts. If any of these methods are incorporated in future clinical applications, then raw data need to be extracted from the scanner after the measurements in addition to traditional file formats.

As previously described, MEGA editing can be used for more than just GABA quantification. For example, 2-hydroxyglutarate which reflects IDH-mutation in gliomas, could also be edited and quantified using the same sequence, which opens up for more interesting research applications. In addition, new editing methods (HERMES) for simultaneous quantification of GABA and glutathione (reflecting redox conditions), would also be interesting to explore further.

The MRSI sequence could have high clinical importance in future applications, although it is a challenging sequence that is limited by several technical challenges. We are currently working on solutions for most of the issues. At this point, we are in the process of increasing the maximal available B_1 field to at least $20 \mu\text{T}$ using phased array coils in order to decrease the echo time and increase the efficiency of the editing. In addition, we are also working on a memory solution with GyroTools, which would make it possible to extend the measurements to 3D or to use phase array coils, without limitations in the reconstruction of spirals. Finally, after resolving the limiting issues, the long-term aim is to apply this sequence *in vivo* in research applications in which small voxel sizes and short acquisition times are necessary.

6. CONCLUSIONS

6.1 Clinical Applications

- I. The trend-level association between MRS concentrations and increased deactivation within the DMN during a working memory task in narcolepsy, supported the theory of an imbalance or misallocation of cognitive resources in favor of maintaining and monitoring sustained attention levels. Since we did not observe any evidence of a true working memory deficit in narcolepsy, the self-reported cognitive difficulties may stem from a dysregulation in the sustained attention system.
- II. We observed a significantly higher mPFC GABA+ concentration in IBS patients with a high severity of comorbid anxiety. This mPFC inhibitory disruption in combination with an aberrant mPFC – ACC connectivity may potentially cause the increased anxiety severity in IBS. However, further multimodal research approaches are needed to shed light on the complex interplay of central and peripheral mechanisms at the interface of neuro-gastroenterology and psychiatry.
- III. A positive correlation between cerebellar GABA+/Glx ratios and tremor severity (ETRS) was observed in patients with severe essential tremor examined prior to DBS surgery. This correlation was mainly driven by a decreased Glx concentration, which suggests that a higher tremor severity in ET may be partly due to a disturbance in cerebellar excitatory neurotransmission. Furthermore, this may in turn be interpreted as a reduced excitatory effect on the inhibitory Purkinje cells, which subsequently results in a decreased inhibitory effect on the dentate nucleus that drives the tremor.

6.1.1 Challenges in the Clinical Setting

Several challenges were encountered, such as time constraints, large voxel sizes, and protocol design, when performing SVS MEGA-PRESS in the clinical research settings. In addition, artifacts in the MRS data originating, for example, from motions, negatively impacted the resulting averaged spectra, which was evident in both data from clinical populations and healthy controls. Therefore, further development and investigation of the methodological approach for GABA detection was necessary in order to meet these common clinical demands.

6.2 Method Development

- IV. All methodologies underestimated the resulting concentration estimates when movements affected the data. OSF performed well compared to standard averaging regardless of whether or not motion-induced artifacts were present in the data. A trend for an improvement was observed for the most intense singlet resonances when using OSF. Finally, rank-ordering the individual phase cycle steps was a slightly better approach than rank-ordering the dynamics.
- V. The implemented JKC method correctly eliminated the majority of the artifact-influenced spectra, and the concentration reliability was further improved compared to OSF. Also, our interpretation was that the JKC method can also be used as a generally applicable retrospective technique for the quality control of a dataset, or as an indication of whether a shift in voxel placement occurred during the measurement.
- VI. Using MEGA-sLASER with a spiral readout developed for a conventional 3 T clinical scanner, our phantom measurements showed that GABA was detectable using a 1:45 min acquisition time and an MRSI voxel size of 1 mL. However, there are several technical challenges, including detrimental effects from eddy currents, spurious echoes, and field inhomogeneities. Even though there are many challenges, an optimized version of this sequence would be extremely valuable in clinical research applications where high spatial resolution and short acquisition times are highly desired.

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