Neurotransmitters & Networks

An MR view on epilepsy and antiepileptic drugs

Printed by Gildeprint, Enschede
Cover photo by Huang Yigao, https://unsplash.com/@ikarishinjigao
ISBN: 978-94-6233-644-5

Printing of this thesis was financially supported by Maastricht University and UCB Pharma BV.

Neurotransmitters & Networks

An MR view on epilepsy and antiepileptic drugs

PROEFSCHRIFT

ter verkrijging van de graad van doctor aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. dr. Rianne M. Letschert
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 13 juli 2017 om 16.00 uur

door

Tamar Marije van Veenendaal

Geboren 6 maart 1988 te Nijkerk

Promotores:

Prof. dr. ir. W.H. Backes
Prof. dr. A.P. Aldenkamp

Copromotor:

Dr. J.F.A. Jansen

Beoordelingscommissie:

Prof. dr. F.M. Mottaghy (voorzitter)
Prof. dr. E. Achten (Universiteit Gent)
Prof. dr. R.M. Dijkhuizen (Universitair Medisch Centrum Utrecht)
Prof. dr. H.J.M. Majoie
Prof. dr. R.J. van Oostenbrugge

Contents

Chapter 1

General introduction

1

Epilepsy

Epilepsy is a neurological disease which is characterized by unprovoked recurrent seizures, during which the brain shows abnormal and excessive neuronal activity. Causes of epilepsy can be manifold and epilepsy is often distinguished into focal and generalized epilepsy. In focal epilepsy, seizures are caused by focal pathological changes, such as cortical malformations or tumors, while in generalized epilepsy, the seizure threshold is lowered throughout the cortex, often due to genetic defects [1].

A large majority of the patients takes antiepileptic drugs (AEDs) to suppress epileptic seizures [2]. More than twenty different AEDs are currently available which can be used in mono-therapy or in combination (poly-therapy). Other treatment options include neurostimulation such as vagal nerve or deep brain stimulation and the ketogenic diet. Surgical resection of the epileptogenic focus is currently the only treatment to cure epilepsy, albeit very invasive and only possible in a subgroup of patients: those with a localized and known epileptic focus [1].

Epilepsy is often accompanied with cognitive and behavioral problems, such as memory or attentional problems [3, 4]. Cognitive problems might originate in the epilepsy itself (i.e. the seizures or the underlying neuropathology), but also medicinal treatment is known to induce cognitive and behavioral side effects [5, 6].

Using validated screening methods, side effects have been reported in 60-90% of the patients with epilepsy [7]. These side effects are an important factor in the discontinuation of AED treatment [8]. Cognitive side effects, such as mental slowing, are among the most reported side effects [2, 9], but the occurrence and severity vary between the different AED types [10].

MR techniques in epilepsy

Several (imaging) techniques are available to study *in vivo* the effects of epilepsy and epileptic seizures on the brain, such as electroencephalography (EEG), positron emission tomography (PET), and magnetic resonance (MR) imaging and spectroscopy [11]. Magnetic resonance encompasses a variety of techniques that employ nuclear magnetic resonance to assess structural, functional, or chemical properties of tissue. By applying different settings, various tissue contrasts with different information can be obtained.

With MR imaging (MRI), magnetic properties of 1H-atoms are manipulated to acquire images, while with MR spectroscopy (MRS), chemical information (of organic molecules) is obtained from a local region. MRS enables concentration measurement of different neurometabolites (Table 1.1). Information about brain function can for instance be obtained with task-related functional MRI. Functional

Table 1.1. Commonly detected neurometabolites [14, 15]. The exact function of most metabolites is still not completely understood and metabolites can have other, unknown functions.

Neurometabolite	Function	Marker for
γ-aminobutyric acid (GABA)	Inhibitory neurotransmitter	Level of tonic inhibition (i.e. constant inhibitory activity)
Choline (Cho)	Component of cell membranes	Neurodegeneration/ inflammation, total membrane content
Creatine (Cr)	Storage form of energy (ATP buffer)	Energy metabolism
Glutamate	Excitatory neurotransmitter, involved in glucose metabolism	Metabolic activity
myo-Inositol (mI)	Osmolyte, Involved in cell growth, storage form of glucose, not well understood	Osmotic stress/edema, neurodegeneration
N-acetyl aspartate (NAA)	Osmolyte, not well understood	Neuronal density, neuronal integrity

MRI measures blood oxygen levels, and thereby indirectly brain activity when performing a task or receiving stimuli. By comparing this activity with and without a task or stimulus, areas involved in this task or stimulus perception can be distinguished.

Both MRI and MRS are currently being applied in patients with epilepsy. MRI is commonly applied during clinical diagnosis of patients, for instance to find (structurally visible) epileptogenic lesions [12]. Both MRS and fMRI are mainly used for research purposes. Clinically, MRS is being applied in the diagnosis of metabolic syndromes and tumor characterizations [13], while fMRI can be used to map specific brain functions to aid the pre-surgical planning [12].

Brain connectivity

The human brain consists of more than 10^{11} neurons and 10^{14} connections, called synapses, linking these neurons [16]. If a neuron is active, an action potential travels through the neuron and when this action potential reaches the synapse, neurotransmitters are released (Figure 1.1). These neurotransmitters induce a signal in the connected neuron, which can in- or decrease the probability of an action potential in that neuron. Several chemical substances may act as neurotransmitters, including glutamate and γ-aminobutyric acid (GABA), which are the most abundant excitatory and inhibitory neurotransmitters in the central nervous system, respectively. Other neurotransmitters are important in several brain diseases but are less abundant, such as dopamine, which is affected in Parkinson's disease, or serotonin, important in mood disorders [16].

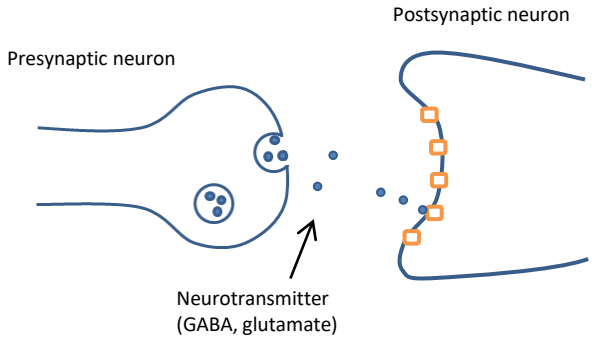

Figure 1.1. Basic mechanism in a synapse. If the presynaptic neuron is active, neurotransmitters are released. Neurotransmitters bind to receptors at the postsynaptic neuron, inducing a signal in that neuron.

Epilepsy, including focal epilepsy, is hypothesized to be a 'network disease', indicating that not one single region is involved in seizure generation and propagation, but rather that it involves the interplay between different brain regions [17, 18]. Epileptic seizures are hypothesized to result from disbalances within these neuronal networks, either because of increased (or decreased) excitatory (or inhibitory) neurotransmission, changes in connectivity, or altered neuronal cell properties [1]. AED treatment targets the dynamic processes that affect this disbalance [19].

Because brain regions are connected, seizure activity might also affect brain structures distant from the seizure focus, even when no seizure activity is present in these structures. Altered brain networks in patients with epilepsy can also explain some of the cognitive problems of these patients, since these networks enable the integration of information, which is essential for complex brain functions [20].

Advanced MR acquisition techniques and analyses methods have been developed which enable assessment of brain networks, for instance fMRI. Besides evaluating brain activity, fMRI can also be used to assess functional connectivity [21, 22]. This connectivity is defined as correlated brain activity, i.e. 'neurons that fire together, wire together' [23]. Several studies have shown a disrupted functional connectivity and organization of brain networks in patients with epilepsy [19], which furthermore appeared to be associated with cognitive decline in epilepsy [21, 22]. However, medication-effects were out of scope in most of these studies, while AED treatment is also an important cause of cognitive problems in patients with epilepsy [5]. In the first part of this thesis, we therefore focused on these medication effects.

Aim and outline of this thesis

The aim of this thesis was to further assess associations of brain connectivity and cognitive problems in epilepsy. Therefore, two goals were formulated:

1. To identify neuronal substrates of cognitive side effects of antiepileptic drugs using magnetic resonance;
2. To explore and evaluate novel MR techniques that may give new insights into epilepsy.

The first, clinical question is described in the first part of this thesis. In Chapter 2, a literature study is presented that elaborates on AEDs in relationship to MR imaging: what are the problems with these drugs, and which possibilities can MR provide to give insights in these problems? Chapter 3 and 4 present the results of an MR study we performed to assess associations between AED use, cognitive problems, and MR biomarkers in patients with chronic epilepsy. In Chapter 3, associations with neurotransmitter levels measured with MRS are described, while Chapter 4 describes the associations with functional brain networks measures.

The second part consists of studies of a more methodological nature. Chapter 5 describes and compares two commonly applied MRS methods to measure brain glutamate levels: PRESS and MEGA-PRESS. This chapter presents the accuracy (tested in a phantom experiment), repeatability (evaluated in human participants) and the concordance between the methods (also tested in human participants). In Chapter 6, an ultra-high-field MRS pilot study is described that assesses the spatial coherence between local neurotransmitter concentrations in both healthy volunteers and patients with epilepsy. This chapter presents the concept of 'neurotransmitter networks', a new method to study brain connectivity on the basis of spatial relations in neurontransmitter concentration distributions.

Finally, all results are summarized and discussed in Chapter 7. This chapter also includes recommendations for further research and is finalized with a general conclusion.

References

[1] J. S. Duncan, J. W. Sander, S. M. Sisodiya, and M. C. Walker. Adult epilepsy. *The Lancet*, 367 (9516):1087–1100, 2006.

[2] R. S. Fisher, B. G. Vickrey, P. Gibson, B. Hermann, P. Penovich, A. Scherer, and S. Walker. The impact of epilepsy from the patient's perspective II: views about therapy and health care. *Epilepsy Research*, 41(1):53–62, 2000.

[3] R. S. Fisher, B. G. Vickrey, P. Gibson, B. Hermann, P. Penovich, A. Scherer, and S. Walker. The impact of epilepsy from the patient's perspective I. descriptions and subjective perceptions. *Epilepsy research*, 41(1):39–51, 2000.

[4] C. E. Elger, C. Helmstaedter, and M. Kurthen. Chronic epilepsy and cognition. *The Lancet Neurology*, 3(11):663–672, 2004.

[5] P. Kwan and M. J. Brodie. Neuropsychological effects of epilepsy and antiepileptic drugs. *The Lancet*, 357(9251):216–222, 2001.

[6] B. Hermann, K. J. Meador, W. D. Gaillard, and J. A. Cramer. Cognition across the lifespan: antiepileptic drugs, epilepsy, or both? *Epilepsy Behav*, 17(1):1–5, 2010.

[7] P. Perucca and F. G. Gilliam. Adverse effects of antiepileptic drugs. *The Lancet Neurology*, 11 (9):792–802, 2012.

[8] H. P. Bootsma, L. Ricker, Y. A. Hekster, J. Hulsman, D. Lambrechts, M. Majoie, A. Schellekens, M. de Krom, and A. P. Aldenkamp. The impact of side effects on long-term retention in three new antiepileptic drugs. *Seizure*, 18(5):327–331, 2009.

[9] S. G. Uijl, C. S. Uiterwaal, A. P. Aldenkamp, J. A. Carpay, J. C. Doelman, K. Keizer, C. J. Vecht, M. C. de Krom, and C. A. van Donselaar. A cross-sectional study of subjective complaints in patients with epilepsy who seem to be well-controlled with anti-epileptic drugs. *Seizure*, 15(4): 242–8, 2006.

[10] D. M. IJff and A. P. Aldenkamp. *Comorbidities of treatment with antiepileptic drugs*, pages 424–36. New York: McGraw-Hill Professional, 2012.

[11] M. Richardson. Current themes in neuroimaging of epilepsy: brain networks, dynamic phenomena, and clinical relevance. *Clin Neurophysiol*, 121(8):1153–75, 2010.

[12] E. Achten. *Het gebruik van medische beeldvorming bij epilepsie*, pages 25–36. Diagnosis Uitgevers, Leusden, 2015.

[13] G. Öz, J. R. Alger, P. B. Barker, R. Bartha, A. Bizzi, C. Boesch, P. J. Bolan, K. M. Brindle, C. Cudalbu, and A. Dinçer. Clinical proton MR spectroscopy in central nervous system disorders. *Radiology*, 270(3):658–679, 2014.

[14] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[15] V. Govindaraju, K. Young, and A. A. Maudsley. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine*, 13(3):129–153, 2000.

[16] E. R. Kandel, J. H. Schwartz, and T. M. Jessell. *Elementary interactions between neurons: synaptic transmission*, volume 4, pages 173 – 308. McGraw-hill New York, 2000.

[17] S. S. Spencer. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*, 43(3):219–227, 2002.

[18] B. C. Bernhardt, L. Bonilha, and D. W. Gross. Network analysis for a network disorder: The emerging role of graph theory in the study of epilepsy. *Epilepsy Behav*, 50:162–70, 2015.

[19] M. A. Rogawski and W. Loscher. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*, 5 (7):553–64, 2004.

[20] H. J. Park and K. Friston. Structural and functional brain networks: from connections to cognition. *Science*, 342(6158):1238411, 2013.

[21] M. P. van den Heuvel and H. E. Hulshoff Pol. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol*, 20(8):519–34, 2010.

[22] S. M. Smith. The future of fMRI connectivity. *NeuroImage*, 62(2):1257–66, 2012.

[23] S. Lowel and W. Singer. Selection of intrinsic horizontal connections in the visual cortex by correlated neuronal activity. *Science*, 255(5041):209, 1992.

Part I

Clinical studies

Chapter 2

Metabolic and functional MR biomarkers of antiepileptic drug effectiveness

T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, P. A. M. Hofman, M. C. G. Vlooswijk, R. P. W. Rouhl, A. J. A. de Louw, W. H. Backes, J. F. A. Jansen, *Neuroscience and Biobehavioral Reviews 2015; 59: 92–99*, DOI: 10.1016/j.neubiorev.2015.10.004

Abstract

As a large number of patients with epilepsy do not respond favorably to antiepileptic drugs (AEDs), a better understanding of treatment failure and the cause of adverse side effects is required. The working mechanisms of AEDs also alter neurotransmitter concentrations and brain activity, which can be measured using MR spectroscopy and functional MR imaging, respectively. This review presents an overview of clinical research of MR spectroscopy and functional MR imaging studies to the effects of AEDs on the brain. Despite the scarcity of studies associating MR findings to the effectiveness of AEDs, the current research shows clear potential regarding this matter. Several GABAergic AEDs have been shown to increase the GABA concentration, which was related to seizure reductions, while language problems due to topiramate have been associated with altered activation patterns measured with functional MR imaging. MR spectroscopy and functional MR imaging provide biomarkers that may predict individual treatment outcomes, and enable the assessment of mechanisms of treatment failure and cognitive side effects.

Introduction

Despite the introduction of several antiepileptic drugs (AEDs) in recent decades, a large number of patients with epilepsy do not respond favorably to AEDs. Ideally, AED usage results in complete seizure freedom, without any unwanted side effects. However, 20-30% of the patients are drug resistant (or, synonymous, medically refractory), i.e. they do not reach seizure freedom after two adequately chosen, dosed and tolerated AEDs [1]. Furthermore, many patients suffer from unwanted side effects, even though the newer generation AEDs is suggested by pharmaceutical companies to have a more beneficial tolerability profile. Today, approximately 10-40% of the patients with epilepsy report side effects spontaneously or in (unstructured) interviews, while 60-90% of patients have been reported to suffer from side effects using validated screening methods [2]. The low effectiveness, i.e. the low combined efficacy and tolerability, results in early treatment discontinuation, high disease burden, and increased health care costs [2, 3]. Considering the need for improvement of the effectiveness, a better understanding of the mechanisms of drug resistance and the cause of side effects is highly desired. This knowledge might, in future, aid the realization of an objective, tailored choice of a specific AED in the individual patient.

Knowledge of the *in vivo* working mechanisms of AEDs is essential to understand drug resistance and side effects. Currently, most AEDs are discovered using animal screening models, in which the anticonvulsant effect of a compound is tested, prior to the exploration of the precise mechanisms of action [1, 4]. Animal models are used to test for efficacy [1] and side effects [5]. The molecular mechanisms of action are assessed at a later stage using *in vitro* research, including studies in neuronal cell cultures, patch-clamp measurements, and biochemistry [6]. These studies have resulted in some basic understanding of the different molecular mechanisms of the available AEDs. However, it still remains difficult to relate these mechanisms of action to the anticonvulsant effect of an AED in patients, mainly because the anticonvulsant effect is also influenced by the pharmacokinetic properties of the compound, such as its ability to cross the blood brain barrier or its metabolism [4, 7]. Furthermore, the complexity of the brain limits a straightforward translation of *in vitro* effects of isolated neuronal cells to *in vivo* effects. Finally, *in vitro* or animal studies cannot completely assess potential side effects on cognition, which can only present themselves after administration in human subjects. Therefore, there is a need for alternative techniques that can assess the effects of AEDs on human brains.

Clinically, magnetic resonance imaging (MRI) is commonly used to provide anatomical information. These anatomical scans are also applied to assess effects

of AEDs on for instance brain volume or cortical thickness [8, 9]. However, there are also MR techniques available that provide information beyond the anatomy, such as metabolism and function, which expectedly are more sensitive to AED treatment. MR spectroscopy (MRS) enables *in vivo* measurements of neurotransmitter and other brain metabolite concentrations, and can therefore be employed to gather insight in the metabolism of AEDs [10, 11]. Another technique is functional MRI (fMRI), which can provide a measure of drug effects on brain activity [12]. These MRI assessments are noninvasive, which makes them suitable for repeated measurements, as no contrast agents or ionizing radiation are necessary, in contrast to other imaging techniques such as computed tomography (CT) or positron emission tomography (PET).

An overview of the previous MRS and fMRI studies to the effects of AEDs on the brain is presented in this review. These MR techniques are sensitive to brain metabolism and function, respectively, which are both directly related to the AED mechanisms of action. Special attention is paid to the possible relation of MR measures with drug resistance and central nervous system (CNS) mediated side effects of AEDs.

Methods

A literature study was performed in Medline/PubMed on August 7, 2015, using the Medical Subject Headings (MeSH) 'anticonvulsants', 'Magnetic Resonance Spectroscopy' and the terms 'functional MRI' or 'fMRI' and 'treatment failure'. Additionally, separate searches were performed per individual AED (the considered AEDs are listed in Table 2.1).Furthermore, relevant references from the reviewed articles are included. Only articles written in English and performed in human subjects are considered. The abstracts of the resulting articles were screened to select only the relevant articles, i.e. articles describing effects of AEDs detectable with MRI, or relating MRI outcomes to seizure reduction or side effects. Studies describing the effects of AEDs in patients with other types of disorders than epilepsy were omitted, because of potential differences in working mechanisms in different diseases, and unknown effects of comorbidities. Studies comparing patients with epilepsy using AEDs to healthy controls not using these AEDs were also omitted, because the effect of the epilepsy itself and drug use cannot be distinguished in these studies.

Table 2.1. Molecular targets of anti-epileptic drugs [13–17].

	Voltage-gated ion channels[a]	Neurotransmitter systems[a]
Benzodiazepines:		GABA system
Clobazam		
Clonazepam		
Midazolam		
Diazepam		
Carbamazepine	Na^+	
Ethosuximide	(Na^+), Ca_2^+	
Felbamate	Na^+, Ca_2^+	GABA system, glutamate receptors
Gabapentin	(Na^+, Ca_2^+)	GABA system
Lacosamide	(Na^+)	Glutamate receptors
Lamotrigine	Na^+, (Ca_2^+)	
Levetiracetam	Ca_2^+	(GABA system, glutamate receptors)
Oxcarbazepine	Na^+, (Ca_2^+, K^+)	
Phenobarbital	Ca_2^+	GABA system, (glutamate receptors)
Phenytoin	Na^+	
Pregabalin	Ca_2^+	
Retigabine	K^+	GABA system
Stiripentol		GABA system
Tiagabine		GABA system
Topiramate	Na^+, Ca_2^+	GABA system, glutamate receptors
Valproate	(Na^+, Ca_2^+)	GABA system, glutamate receptors
Vigabatrin		GABA system
Zonisamide	Na^+, Ca_2^+	

[a]Not all molecular mechanisms are well understood; possible molecular targets are displayed between parentheses.

Pharmacodynamic and pharmacokinetic mechanisms of action

Epileptic seizures are characterized by excessive, synchronal neuronal activity in the brain. AEDs, ideally, suppress this activity via several distinct mechanisms, which can be divided into three main categories: 1) Modulation of the voltage-gated ion channels, i.e. of the sodium or calcium channels, and, less common, of the potassium channels. This modulation can result in a more stable membrane potential, reduced release of neurotransmitters, and a reduction in seizure spread. The exact effects of this modulation depend on the specific channel type. 2) Elevation of the seizure threshold by targeting the γ-aminobutyric acid (GABA) system. The GABA system can be affected by two mechanisms: by increasing the sensitivity of the $GABA_A$ receptors or by augmenting the GABA concentration. 3) Reduction of the excitatory neurotransmission. AEDs with the latter mechanism

function as antagonists of the glutamate receptors [15, 17]. Several AEDs combine different mechanisms, and the mechanisms are not completely understood for all AEDs (Table 2.1).

In addition to these pharmacodynamic mechanisms, pharmacokinetic properties contribute greatly to the positive and negative effects of AEDs. Pharmacokinetic properties include the absorption, distribution, metabolism, and excretion of a compound. These factors can differ among different users and with different AEDs, and complicate the prediction of the efficacy and the tolerability in individual patients [18].

Unfortunately, the mechanisms that aim to suppress epileptic seizures can also affect normal brain activity. Modifying these mechanisms can also induce side effects. These CNS mediated side effects include sedation, coordination disturbances, cognitive difficulties, and behavioral problems. Although the probability and severity of these side effects depend on the AED type, several of these events are quite similar among the different AEDs [2]. Also several non-CNS mediated side effects can occur with the use of AEDs, which might result from pharmacokinetic properties, interaction effects, or effects on other organ systems and the immune system (allergic reactions) [2, 3].

Treatment failure is defined as the appearance of recurrent seizures after adequate intervention [19]. Treatment failure in previous AEDs is a strong indicator of treatment failure for new AEDs: While approximately 62% of the patients became seizure free after the first, adequately chosen AED, only 17% of the patients became seizure free after failure of two to five adequately chosen AEDs in a cohort study of 478 patients with newly administered AEDs and various epilepsy types [20]. The mechanisms of treatment failure and drug resistance are also still largely unknown, and both pharmacodynamic and pharmacokinetic properties are likely to be involved in these mechanisms. Several hypotheses have been formulated which explain treatment failure in patients with epilepsy, of which the transporter and the target hypothesis are most popular. The transporter hypothesis argues that AEDs cannot sufficiently penetrate the epileptogenic brain tissue, as a result of increased expression of efflux transporters in the blood-brain barrier. According to the target hypothesis, AEDs are able to reach the ion channels or receptors, but cannot exert their function due to structural and/or functional alterations of these targets. A combination of the above listed hypotheses and other mechanisms (including inflammatory, epigenetic, and also unknown pathways) most likely cooperate in some way to drug resistance in epilepsy [1, 21].

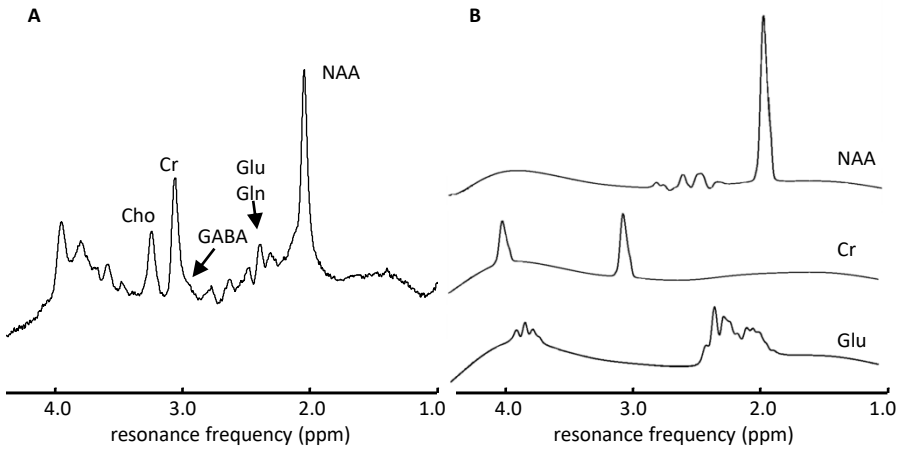

Figure 2.1. A. Example of magnetic resonance spectrum measured in the occipital lobe of a healthy human. The concentrations of NAA, creatine and the choline-containing compounds are relatively high in the brain, resulting in large resonance peaks. Although GABA, glutamate and glutamine are also abundant in the brain, their resonance peaks are much smaller because of spin-spin interactions. The $4\,CH_2$ group of GABA has approximately the same resonance frequency as the $N(CH_3)$ group of creatine and is only measurable using advanced acquisition or analysis methods [22]. The same holds for glutamate and glutamine. B. Estimations by LCModel [23] of the contribution of single molecules to the spectrum displayed in A. Cho: choline-containing compounds; Cr: creatine; GABA: γ-aminobutyric acid; Glu: glutamate; Gln: glutamine; NAA: N-acetyl-aspartate; ppm: parts per million.

Magnetic resonance spectroscopy

AEDs exert their anticonvulsant properties by affecting the excitatory and inhibitory neurotransmitter systems. Effects on the GABA and glutamate concentrations can be measured directly *in vivo* using ^{1}H-MRS (proton MRS). The merit of ^{1}H-MRS is based on the shielding effect of the chemical environment of protons, which causes a small shift in resonance frequency. ^{1}H-MRS results in a spectrum with peaks at different resonance frequencies, characteristic for different molecule groups (Figure 2.1). The area underneath these peaks is proportional to the concentration of the molecule. Whether it is possible to measure a particular metabolite depends on its concentration in the brain, spectral overlap with other metabolites, and spin-spin interactions, which can result in lower resonance peaks [10].

GABA and glutamate both are subject to spectral overlap and spin-spin interactions (Figure 2.1). Special editing techniques or 2D ^{1}H-MRS are commonly used to resolve this problem [10, 24]. These techniques enable a reliable estimation of the concentration of the metabolites [24]. Due to their overlapping resonances,

the glutamate and glutamine concentrations are frequently combined, resulting in a so-called 'Glx' concentration. Other metabolites which are commonly measured using ^{1}H-MRS include N-acetyl aspartate (NAA), creatine or choline-containing compounds. The functions of these metabolites are elaborately discussed elsewhere [22, 25].

Several AEDs with a GABAergic mechanism of action have been shown to elevate GABA concentrations in the brain, including vigabatrin (VGB) [26–32], topiramate (TPM) [33–36] and gabapentin (GBP) [34, 37–39] (Figure 2.2). Elevated GABA concentrations were already detectable within hours after intake of a single dose and also appeared during chronic VGB, TPM and GBP use, although no effects of a single low dose GBP on the GABA concentration were found by Preuss et al. [40]. There is a linear relation between the VGB dosage and the resulting GABA concentration. However, with high VGB dosages, the GABA concentration does not increase any further and reaches a plateau [41]. The GABA concentration was related to seizure reduction in patients with focal epilepsy with VGB [42] and GBP use [39]. Moreover, a relation was found between seizure reduction and the GABA concentration before and during VGB treatment in patients with poorly controlled focal epilepsy [43]. Patients with focal epilepsy with complete seizure control had a lower baseline GABA concentration in the epileptic hemisphere, compared with the non-epileptic hemisphere, and a significant increase of GABA. However, patients with no VGB-induced seizure reduction did not reveal concentration differences between the hemispheres or a significant increase in GABA concentration. The pretherapeutic concentration differences between the hemispheres correlated with the seizure reduction during VGB treatment [44]. These results suggest a causal relation between GABAergic mechanisms of action, the increase in GABA concentration, and seizure reduction. However, this causal link cannot be proven using these radiological techniques in human studies.

In contrast to these results, no effects of a single dose of tiagabine (TGB) on the GABA concentration were found in healthy participants by Myers et al. [45], although TGB is an AED with a solely GABAergic mechanism of action as well. Generalizations across the different AEDs should be considered with caution, because the mechanism of action each individual AED is unique even for chemically related AEDs.

The effects of levetiracetam (LEV) on the GABA concentration are not clear. The mechanisms of action of LEV are not completely understood but according to literature it has probably multiple mechanisms of action, including a GABAergic mechanism [15]. While one study showed a significant increase in the GABA concentration after LEV use in patients with focal epilepsy who had a seizure reduction [46], another study failed to show effects on the GABA concentration in

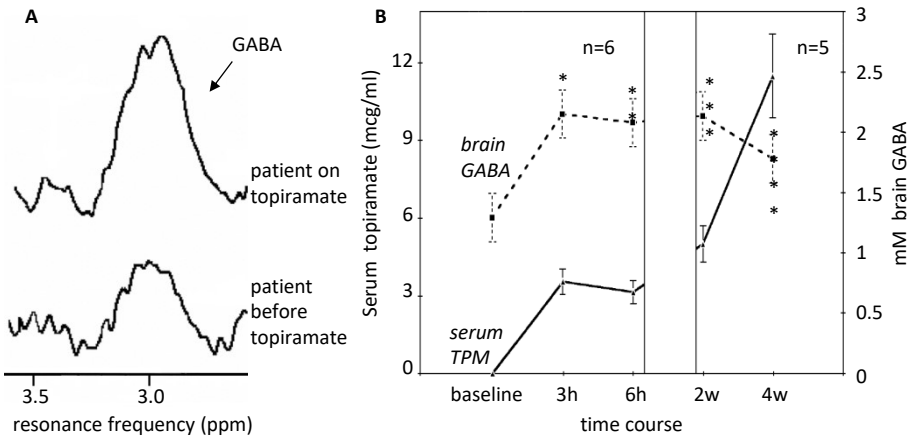

Figure 2.2. Graphs illustrating the effect of topiramate on the GABA concentration, as measured with MR spectroscopy. A. Serial GABA spectra of a patient with epilepsy before and after use of topiramate. This spectrum, measured using a special editing technique, shows an increase in GABA concentration in an individual patient [Reprinted from Petroff et al. [35]]. B. Time changes of the GABA concentrations (dashed line) and serum topiramate (TPM, solid line) levels before and during TPM use [Reprinted from Kuzniecky et al. [34]]. The GABA concentration is significantly increased compared with the baseline concentration during TPM use. *: $p<0.001$, **: $p<0.006$, ***: $p<0.002$, ****: $p<0.005$. GABA: γ-aminobutyric acid; ppm: parts per million, h: hours, w: weeks

healthy participants [47]. Besides the difference in participants (patients with focal epilepsy versus healthy people), this difference might be explained by the timing of the measurements: the MRS measurements were performed before and 2-6 weeks after initiation of LEV treatment in the patients with epilepsy, while the measurements were performed before, at 3 and at 6 hours after a single dose of LEV in the healthy participants. The effect on the GABA concentration of AEDs without a known GABAergic mechanism of action is rarely assessed. However, elevated GABA concentrations are shown in healthy participants with lamotrigine (LTG) use, albeit only after four weeks use and not directly after a single dose or after 2 weeks of LTG usage, implying that the GABA concentration is increased by indirect effects of LTG [34].

Also the effects of AEDs on the homocarnosine and pyrrolidine metabolites were assessed. These metabolites are precursors of GABA and have been suggested to have anticonvulsant properties themselves [48]. The homocarnosine and pyrrolidine concentrations were shown to increase with the use of TPM [35, 36], VGB [28, 49] or GBP [38]. The authors suggested that homocarnosine (and not GABA) is associated with seizure reduction in patients with focal epilepsy using TPM and GBP [50], using VGB [49], or in a group of patients with focal epilepsy or juvenile

myoclonic epilepsy using valproate (VPA) or LTG [51].

The effects of AEDs on the glutamate concentration are also not clear. Articles assessing the effects of GBP [37, 40], benzodiazepines [52, 53], VPA [54], or TPM [55] on the glutamate, glutamine, or Glx concentration failed to show consistent results. In contrast to the GABAergic mechanisms, AEDs do not alter the glutamate concentrations directly, but rather decrease the sensitivity of the glutamate receptors. The glutamate concentration might be decreased through the negative modulation of the voltage-gated channels, which are affected by most of the AEDs.

Indirectly, AEDs might also affect the concentrations of other metabolites. Campos et al. [56] showed decreased NAA concentrations in patients with focal epilepsy who did not respond to their AED treatment compared with responders one to two years after initiation of AED therapy. As the NAA concentration is generally associated with neuronal density or integrity, these results suggest that treatment failure can be associated with neuronal damage. The choline concentration did not differ between these responder groups. Furthermore, patients with various types of epilepsy using VPA showed reduced myo-inositol concentrations compared with patients taking other AEDs, but similar NAA and creatine concentrations [54, 57]. The authors argue that the myoinositol reductions are not likely to be related to the antiepileptic efficacy of VPA. In healthy participants, no changes in the NAA or choline concentrations were measured after GBP intake [40], and also lorazepam intake did not affect the NAA, creatine, myoinositol, or trimethylamine concentrations [52].

Effects on neurotransmitter concentrations can also be measured in animal models. However, the results of animal studies do not always correspond to human studies. For instance, the effect of TPM on the GABA concentration was not predicted by animal models [35]. Moreover, homocarnosine concentrations are much lower in rodents compared to humans, while homocarnosine is suggested to be involved in the anticonvulsant mechanisms [35]. The results of Kuzniecky et al. [34], showing long-term elevations of GABA after LTG use, illustrate that also AEDs without a known GABAergic mechanism can (indirectly) alter the GABA concentrations. This necessitates human *in vivo* measurements.

Functional MRI

fMRI uses the blood oxygen level dependent (BOLD) effect to indirectly measure brain activity. By comparing the BOLD signal of a baseline condition to a situation with a task, an activity measure for the brain areas involved in this task can be obtained [12]. BOLD measurements can also be performed without a certain

task. This so-called resting state fMRI measures the spontaneous fluctuations of the ongoing neural signaling. The spontaneous fluctuations show correlations between several distinct brain areas, and these correlations are assumed to reflect intrinsic functional connections. Advanced analysis techniques, such as independent component analysis or graph theory, can be applied to assess the functional brain connectivity [58].

It is plausible to assume that AEDs, by suppressing the epileptiform activity, also affect normal brain activity and thereby the BOLD signal. Different brain areas might be more susceptible to AED actions compared with other regions, as AEDs exert their function on specific receptors which might be more prominent in specific brain areas than other. fMRI can be used to identify these altered activation patterns in relation to CNS-mediated side effects or treatment failure.

Several studies employing task fMRI indeed show that AEDs have different effects on brain activation patterns in healthy participants [59–65] or in patients with drug resistant temporal lobe epilepsy [66]. These effects vary among AEDs [67, 68] and depend on the specific task performed during the measurements [69]. While AEDs mainly attenuate the activation patterns, as can be expected from their mechanisms of action, also enhanced activation during AED use has been reported [70]. This seemingly contradictory result could be an indirect effect of attenuated activation in other brain areas, or result directly from AED mechanisms, as a computer simulation showed that modulation of the sodium channels by phenytoin or carbamazepine can also lead to increased excitation [71].

Using graph analysis, a lower hubness (the presence of hyperconnected nodes that connect distant parts of the brain) was found in patients with temporal lobe epilepsy using carbamazepine or oxcarbazepine compared with patients using other AEDs, implying a less efficient organization [72]. Relating these findings to anticonvulsant mechanisms or the development of CNS mediated side effects was outside the scope of these articles.

Other studies assessed associations between cognitive side effects, brain activation and TPM, which induces cognitive side effects including language disturbances [14]. In a study comparing patients with cryptogenic (i.e. with unknown cause) focal drug resistant epilepsy using TPM with patients using other AEDs, several language areas appeared to be significantly underactivated during a language task in the patients using TPM (Figure 2.3). Decreased activation in these areas was also correlated to the language problems [73]. Similar results were found in patients with migraine treated with TPM [74]. Another study found comparable differences in brain activation between patients with temporal lobe epilepsy using TPM and patients using other AEDs, although the observed differences also depended on the lateralization of the epileptic focus [75]. Besides effects in lan-

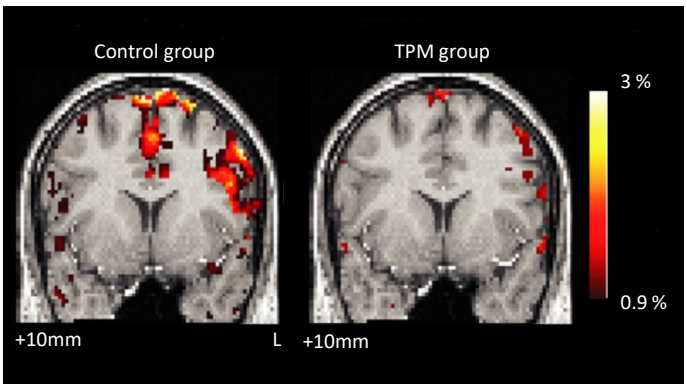

Figure 2.3. Activations maps of a group of patients with cryptogenic focal epilepsy using topiramate (TPM, n=5, right) versus patients using other AEDs (n=10), obtained using a covert word generation paradigm [Reprinted from Jansen et al. [73]]. These activation maps show a significantly underactivation in the language areas in patients using topiramate compared with other patients. L: left.

guage areas, patients with frontal lobe epilepsy taking TPM showed a reduced deactivation of the default mode network [76]. The default mode network consists of functionally connected brain areas which are active during rest, but deactivate during tasks. Appropriate deactivation of this network is considered necessary for correct task performance.

In contrast to TPM, LEV does not negatively affect cognitive abilities, and is even suggested to improve cognitive function [14]. Patients with temporal lobe epilepsy taking LEV showed more deactivations in the ipsilateral mesial temporal structures compared with patients using other AEDs during working memory tasks [77]. Stronger deactivation in these structures is commonly associated with improved functioning [77]. Whether this reduction was also associated with better cognitive functioning in this study was not reported.

Treatment failure is hypothesized to be caused by alterations of the blood-brain barrier or the molecular targets of the AEDs [1]. Because of these alterations, AEDs cannot exert their function in the epileptogenic brain tissue. However, the location of this epileptogenic brain tissue varies largely between patients, while fMRI is mainly employed to assess the susceptibility of distinct brain areas to the effects of AEDs. In case fMRI experiments are combined with knowledge about the epileptic focus, fMRI might provide new information about mechanisms of action.

Interestingly, Kay et al. [78] also showed associations between treatment resistance and functional connectivity. Patients with idiopathic generalized epilepsy resistant to AEDs showed a reduced connectivity in the default mode network

compared with patients who did show a seizure reduction. Whether this is a consequence of the drug resistance (i.e. the continuing seizures) or is preceding the drug resistance remains unknown.

Limitations

The application of MR techniques in AED treatment is still in its infancy, and several research gaps need to be bridged before these techniques can prove their clinical utility. Currently, the number of MR studies assessing the effects of AEDs is limited, and only a few studies relate MR outcomes to either seizure reduction or CNS mediated side effects. Furthermore, many of the included articles have low participant numbers and have to deal with practical constraints such as polytherapy and possible confounding effects of the epilepsy itself, limiting the quality of these studies.

Beside these general study limitations, the different MR techniques also have some limitations. The interpretation of ^{1}H-MRS findings is currently debated [79, 80]. ^{1}H-MRS measures all available neurotransmitters: both synaptically and extrasynaptically (presynaptic terminals, synaptic vesicles or neurotransmitter uptake mechanisms), and it is not known where and how these neurotransmitters act precisely [79]. ^{1}H-MRS is also only able to measure the neurotransmitter concentrations but not the receptor sensitivity, while this sensitivity is affected by AEDs in particular and could be crucial for effectiveness. Furthermore, the neurotransmitter concentrations are usually measured in a large, single voxel located in the occipital brain regions, whereas the majority of the side effects concerns functions dependent on other brain regions. The use of smaller voxels or voxels located in those other brain areas is limited by the signal-to-noise ratio (SNR) and magnetic field inhomogeneities.

Moreover, most AEDs exert their anticonvulsant activity using several mechanisms, and the interaction between these mechanisms is largely unknown. Only analyzing the effects of the AEDs on the GABA concentration might therefore be too simplistic. Although currently not many effects of AEDs on the glutamate concentrations are shown, it is recommended to measure this concentration as well to have an indication of the balance between the main inhibitory and excitatory mechanisms.

No general conclusions can be drawn about the specific effects of AEDs on the brain activity. Most fMRI studies are performed with specific tasks, and the results of these studies are difficult to generalize because of their task-dependency. Another drawback of fMRI studies is that some AEDs might affect the blood flow and thereby the BOLD signal, irrespective of their antiepileptic effect. These effects

should be considered in future fMRI studies to AEDs. For instance, fMRI studies can be combined with arterial spin labeling measurements, to measure the blood flow [81].

Perspectives

Despite these limitations, both MRS and fMRI results show potential promising applications in future AED research. The suggested relation between GABAergic mechanisms of action, the GABA concentration measured using MRS, and seizure reduction implies several possibilities. First, the GABA concentration can be used as a biomarker for seizure reduction, enabling an earlier indication of treatment failure than clinical evaluation. Furthermore, MRS might provide insights in the development of CNS mediated side effects. The mechanisms responsible for this development still remain largely unknown, although some studies hypothesize that GABAergic mechanisms are involved [5, 73]. By providing a tool to measure the effects of these GABAergic mechanisms, MRS might indicate whether these mechanisms can indeed be associated with CNS mediated side effects. Therefore, MRS might provide valuable information for the selection of the most suitable AED, or exclusion of AEDs which are less suitable, prior to or soon after initiation of treatment. These potential utilities can only be proven after future suitable studies.

fMRI might be employed to pinpoint the neuronal substrate of the side effects. Abnormal activation patterns could possibly explain in part why some patients experience more and others less CNS mediated side effects, and might even predict this for individual patients. Furthermore, the occurrence of side effects might be predicted for new antiepileptic compounds, if it is known how AED effects on brain activity are associated with these side effects.

New studies should focus on the usefulness of these MR techniques for different types of epilepsy, especially when relating treatment failure to MR outcome. Treatment failure is more common in specific epilepsy syndromes (Ohtahara syndrome, early myoclonic encephalopathy, West syndrome, Dravet syndrome, or Lennox-Gastaut syndrome) and underlying etiologies (hippocampal sclerosis, cortical dysplasia, hemorrhage) [82], and the mechanisms of drug resistance might depend on the specific brain pathology [21]. MRS and fMRI might also be employed to assess the effects of other epilepsy treatments, such as the ketogenic diet, and compare these to AED effects on brain metabolite concentrations or activation patterns [83].

Novel, and more advanced MR technologies offer new opportunities to overcome many of the current limitations. With the use of higher magnetic field strengths, the SNR of MRS can be increased. Smaller voxels, frontally located voxels, and even multivoxel MRS becomes feasible with 7 Tesla MR studies (see for instance Pan

et al. [84]). These studies can increase the knowledge of regional effects of AEDs on the neurotransmitter concentration. Besides ^{1}H-MRS, also ^{13}C-MRS can be employed. This method enables the assessment of neurotransmitter cycling and human brain energetics, although ^{13}C-MRS is less accessible than ^{1}H-MRS due to the low natural abundance of ^{13}C and the need for labeled compounds and special hardware [85]. Furthermore, while MRS does not measure the receptor sensitivity, multimodal studies combining PET and MRS enable assessments of both receptor sensitivity and neurotransmitter concentrations [32]. Finally, contrast-enhanced MRI allows for the assessment of the blood-brain barrier integrity, which could be an important feature of treatment failure [86].

The advanced analysis methods for fMRI data provide opportunities to assess the functional brain connectivity. This functional brain connectivity might be more related to cognition than brain activity patterns [87], and therefore more relevant to assess especially cognitive side effects than task related activity analysis. These methods are often employed in combination with resting state fMRI, thereby also omitting the task-dependence of the results [58].

To conclude, MR techniques provide several unique possibilities to assess neuronal substrates of the effectiveness of AEDs, which might be employed for future individualized patients care. These possibilities are supported by the technological improvements of the last decade, which open new possibilities to apply fMRI and MRS to assess AED mechanisms and effects. However, future studies are still necessary to investigate the potential of the different MR techniques to provide biomarkers, to predict treatment outcome or to assess the mechanisms of treatment failure and side effects.

References

[1] W. Loscher, H. Klitgaard, R. E. Twyman, and D. Schmidt. New avenues for anti-epileptic drug discovery and development. *Nat Rev Drug Discov*, 12(10):757–76, 2013.

[2] P. Perucca and F. G. Gilliam. Adverse effects of antiepileptic drugs. *The Lancet Neurology*, 11 (9):792–802, 2012.

[3] R. Toledano and A. Gil-Nagel. Adverse effects of antiepileptic drugs. *Semin Neurol*, 28(3):317–27, 2008.

[4] M. A. Rogawski. Molecular targets versus models for new antiepileptic drug discovery. *Epilepsy research*, 68(1):22–28, 2006.

[5] R. Sankar and G. L. Holmes. Mechanisms of action for the commonly used antiepileptic drugs: Relevance to antiepileptic drug–associated neurobehavioral adverse effects. *Journal of Child Neurology*, 19(1 suppl):S6–S14, 2004.

[6] M. A. Dichter and J. Pollard. *Models of Seizures and Epilepsy*, chapter Cell Culture Models for Studying Epilepsy, pages 23–34. Elsevier Academic Press, 2006.

[7] M. J. Brodie, A. Covanis, A. Gil-Nagel, H. Lerche, E. Perucca, G. J. Sills, and H. S. White. Antiepileptic drug therapy: does mechanism of action matter? *Epilepsy & Behavior*, 21(4): 331–41, 2011.

[8] F. A. De Marco, E. Ghizoni, E. Kobayashi, L. M. Li, and F. Cendes. Cerebellar volume and long-term use of phenytoin. *Seizure*, 12(5):312–315, 2003.

[9] H. R. Pardoe, A. T. Berg, and G. D. Jackson. Sodium valproate use is associated with reduced parietal lobe thickness and brain volume. *Neurology*, 80(20):1895–1900, 2013.

[10] N. A. Puts and R. A. Edden. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog Nucl Magn Reson Spectrosc*, 60:29–41, 2012.

[11] A. J. Ross and P. S. Sachdev. Magnetic resonance spectroscopy in cognitive research. *Brain Res Rev*, 44(2-3):83–102, 2004.

[12] R. G. Wise and I. Tracey. The role of fMRI in drug discovery. *J Magn Reson Imaging*, 23(6): 862–76, 2006.

[13] J. L. Fisher. The effects of stiripentol on GABA(A) receptors. *Epilepsia*, 52 Suppl 2:76–8, 2011.

[14] P. Kwan and M. J. Brodie. Neuropsychological effects of epilepsy and antiepileptic drugs. *The Lancet*, 357(9251):216–222, 2001.

[15] M. A. Rogawski and W. Loscher. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*, 5 (7):553–64, 2004.

[16] L. J. Stephen and M. J. Brodie. Pharmacotherapy of epilepsy. *CNS drugs*, 25(2):89–107, 2011.

[17] H. S. White, M. D. Smith, and K. S. Wilcox. Mechanisms of action of antiepileptic drugs. *International review of neurobiology*, 81:85–110, 2007.

[18] C. Johannessen Landmark, S. I. Johannessen, and T. Tomson. Host factors affecting antiepileptic drug delivery-pharmacokinetic variability. *Adv Drug Deliv Rev*, 64(10):896–910, 2012.

[19] P. Kwan, A. Arzimanoglou, A. T. Berg, M. J. Brodie, W. Allen Hauser, G. Mathern, S. L. Moshe, E. Perucca, S. Wiebe, and J. French. Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. *Epilepsia*, 51(6):1069–77, 2010.

[20] Y. Schiller and Y. Najjar. Quantifying the response to antiepileptic drugs effect of past treatment history. *Neurology*, 70(1):54–65, 2008.

[21] D. Schmidt and W. Loscher. Drug resistance in epilepsy: putative neurobiologic and clinical mechanisms. *Epilepsia*, 46(6):858–77, 2005.

[22] V. Govindaraju, K. Young, and A. A. Maudsley. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine*, 13(3):129–153, 2000.

[23] S. W. Provencher. Automatic quantitation of localized in vivo 1H spectra with LCModel. *NMR in Biomedicine*, 14(4):260–264, 2001.

[24] M. E. Henry, T. L. Lauriat, M. Shanahan, P. F. Renshaw, and J. E. Jensen. Accuracy and stability of measuring GABA, glutamate, and glutamine by proton magnetic resonance spectroscopy: a phantom study at 4 Tesla. *J Magn Reson*, 208(2):210–8, 2011.

[25] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[26] S. G. Mueller, O. M. Weber, P. Boesiger, and H. G. Wieser. Influence of pyridoxal 5'-phosphate alone and in combination with vigabatrin on brain GABA measured by 1H-NMR-spectroscopy. *Brain research bulletin*, 55(4):555–560, 2001.

[27] E. J. Novotny, F. Hyder, M. Shevell, and D. L. Rothman. GABA changes with vigabatrin in the developing human brain. *Epilepsia*, 40(4):462–466, 1999.

[28] O. A. C. Petroff, F. Hyder, T. Collins, R. H. Mattson, and D. L. Rothman. Acute effects of vigabatrin on brain GABA and homocarnosine in patients with complex partial seizures. *Epilepsia*, 40(7):958–964, 1999.

[29] O. A. C. Petroff, D. L. Rothman, K. L. Behar, and R. H. Mattson. Initial observations on effect of vigabatrin on in vivo 1H spectroscopic measurements of γ-aminobutyric acid, glutamate, and glutamine in human brain. *Epilepsia*, 36(5):457–464, 1995.

[30] O. A. C. Petroff, D. L. Rothman, K. L. Behar, T. L. Collins, and R. H. Mattson. Human brain GABA levels rise rapidly after initiation of vigabatrin therapy. *Neurology*, 47(6):1567–1571, 1996.

[31] N. P. L. G. Verhoeff, O. A. C. Petroff, F. Hyder, S. S. Zoghbi, M. Fujita, N. Rajeevan, D. L.

Rothman, J. P. Seibyl, R. H. Mattson, and R. B. Innis. Effects of vigabatrin on the GABAergic system as determined by [123I] iomazenil SPECT and GABA MRS. *Epilepsia*, 40(10):1433–1438, 1999.

[32] O. M. Weber, A. Verhagen, C. O. Duc, D. Meier, K. L. Leenders, and P. Boesiger. Effects of vigabatrin intake on brain GABA activity as monitored by spectrally edited magnetic resonance spectroscopy and positron emission tomography. *Magnetic resonance imaging*, 17(3):417–425, 1999.

[33] R. Kuzniecky, H. Hetherington, S. Ho, J. Pan, R. Martin, F. Gilliam, J. Hugg, and E. Faught. Topiramate increases cerebral GABA in healthy humans. *Neurology*, 51(2):627–629, 1998.

[34] R. Kuzniecky, S. Ho, J. Pan, R. Martin, F. Gilliam, E. Faught, and H. Hetherington. Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology*, 58 (3):368–372, 2002.

[35] O. A. C. Petroff, F. Hyder, R. H. Mattson, and D. L. Rothman. Topiramate increases brain GABA, homocarnosine, and pyrrolidinone in patients with epilepsy. *Neurology*, 52(3):473–473, 1999.

[36] O. A. C. Petroff, F. Hyder, D. L. Rothman, and R. H. Mattson. Topiramate rapidly raises brain GABA in epilepsy patients. *Epilepsia*, 42(4):543–548, 2001.

[37] K. Cai, R. P. Nanga, L. Lamprou, C. Schinstine, M. Elliott, H. Hariharan, R. Reddy, and C. N. Epperson. The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T (1)H-MRS study. *Neuropsychopharmacology*, 37(13):2764–71, 2012.

[38] O. A. Petroff, F. Hyder, D. L. Rothman, and R. H. Mattson. Effects of gabapentin on brain GABA, homocarnosine, and pyrrolidinone in epilepsy patients. *Epilepsia*, 41(6):675–80, 2000.

[39] O. A. Petroff, D. L. Rothman, K. L. Behar, D. Lamoureux, and R. H. Mattson. The effect of gabapentin on brain gamma-aminobutyric acid in patients with epilepsy. *Ann Neurol*, 39(1):95–9, 1996.

[40] N. Preuss, J. W. van der Veen, P. J. Carlson, J. Shen, and G. Hasler. Low single dose gabapentin does not affect prefrontal and occipital gamma-aminobutyric acid concentrations. *Eur Neuropsychopharmacol*, 23(12):1708–13, 2013.

[41] O. A. C. Petroff, D. L. Rothman, K. L. Behar, and R. H. Mattson. Human brain GABA levels rise after initiation of vigabatrin therapy but fail to rise further with increasing dose. *Neurology*, 46(5):1459–1459, 1996.

[42] O. A. C. Petroff, K. L. Behar, R. H. Mattson, and D. L. Rothman. Human brain γ-aminobutyric acid levels and seizure control following initiation of vigabatrin therapy. *Journal of neurochemistry*, 67(6):2399–2404, 1996.

[43] S. G. Mueller, O. M. Weber, C. O. Duc, B. Weber, D. Meier, W. Russ, P. Boesiger, and H. G. Wieser. Effects of vigabatrin on brain GABA+/Cr signals in patients with epilepsy monitored by 1H-NMR-spectroscopy: Responder characteristics. *Epilepsia*, 42(1):29–40, 2001.

[44] S. G. Mueller, O. M. Weber, C. O. Duc, D. Meier, W. Russ, P. Boesiger, and H. G. Wieser. Effects of vigabatrin on brain GABA+/Cr signals in focus distant and focus near brain regions monitored by 1H-NMR spectroscopy. *European Journal of Neurology*, 10(1):45–52, 2003.

[45] J. F. Myers, C. J. Evans, N. J. Kalk, R. A. Edden, and A. R. Lingford-Hughes. Measurement of GABA using J-difference edited 1H-MRS following modulation of synaptic GABA concentration with tiagabine. *Synapse*, 68(8):355–62, 2014.

[46] M. T. Doelken, T. Hammen, W. Bogner, A. Mennecke, A. Stadlbauer, U. Boettcher, A. Doerfler, and H. Stefan. Alterations of intracerebral gamma-aminobutyric acid (GABA) levels by titration with levetiracetam in patients with focal epilepsies. *Epilepsia*, 51(8):1477–82, 2010.

[47] R. Kuzniecky, J. Pan, A. Burns, O. Devinsky, and H. Hetherington. Levetiracetam has no acute effects on brain γ-aminobutyric acid levels. *Epilepsy & Behavior*, 12(2):242–244, 2008.

[48] O. A. C. Petroff. Book review: GABA and glutamate in the human brain. *The Neuroscientist*, 8 (6):562–573, 2002.

[49] O. A. C. Petroff, R. H. Mattson, K. L. Behar, F. Hyder, and D. L. Rothman. Vigabatrin increases human brain homocarnosine and improves seizure control. *Annals of neurology*, 44(6):948–952, 1998.

[50] O. A. C. Petroff, F. Hyder, D. L. Rothman, and R. H. Mattson. Brain homocarnosine and seizure control of patients taking gabapentin or topiramate. *Epilepsia*, 47(3):495–498, 2006.

[51] O. A. C. Petroff, F. Hyder, D. L. Rothman, and R. H. Mattson. Homocarnosine and seizure control in juvenile myoclonic epilepsy and complex partial seizures. *Neurology*, 56(6):709–715, 2001.

[52] P. Brambilla, J. A. Stanley, M. Nicoletti, K. Harenski, K. F. Wells, A. G. Mallinger, M. S. Keshavan, and J. C. Soares. 1H MRS brain measures and acute lorazepam administration in healthy human subjects. *Neuropsychopharmacology*, 26:546–551, 2002.

[53] M. E. Henry, J. E. Jensen, S. C. Licata, C. Ravichandran, M. L. Butman, M. Shanahan, T. L. Lauriat, and P. F. Renshaw. The acute and late CNS glutamine response to benzodiazepine challenge: A pilot pharmacokinetic study using proton magnetic resonance spectroscopy. *Psychiatry Research: Neuroimaging*, 184(3):171–176, 2010.

[54] R. J. Simister, M. A. McLean, G. J. Barker, and J. S. Duncan. The effect of sodium valproate on proton MRS visible neurochemical concentrations. *Epilepsy Res*, 74(2-3):215–9, 2007.

[55] C. M. Moore, M. Wardrop, B. Frederick B. de, and P. F. Renshaw. Topiramate raises anterior cingulate cortex glutamine levels in healthy men; a 4.0T magnetic resonance spectroscopy study. *Psychopharmacology (Berl)*, 188(2):236–43, 2006.

[56] B. A. Campos, C. L. Yasuda, G. Castellano, E. Bilevicius, L. M. Li, and F. Cendes. Proton MRS may predict AED response in patients with TLE. *Epilepsia*, 51(5):783–8, 2010.

[57] R. J. Simister, M. A. McLean, G. J. Barker, and J. S. Duncan. Proton MRS reveals frontal lobe metabolite abnormalities in idiopathic generalized epilepsy. *Neurology*, 61(7):897–902, 2003.

[58] M. P. van den Heuvel and H. E. Hulshoff Pol. Exploring the brain network: a review on resting-state fMRI functional connectivity. *Eur Neuropsychopharmacol*, 20(8):519–34, 2010.

[59] R. L. Aupperle, D. Tankersley, L. N. Ravindran, T. Flagan, N. R. Stein, M. B. Stein, and M. P. Paulus. Pregabalin effects on neural response to emotional faces. *Front Hum Neurosci*, 6:42, 2012.

[60] C. M. Del-Ben, C. A. Ferreira, T. A. Sanchez, W. C. Alves-Neto, V. G. Guapo, D. B. de Araujo, and F. G. Graeff. Effects of diazepam on BOLD activation during the processing of aversive faces. *J Psychopharmacol*, 26(4):443–51, 2012.

[61] G. D. Iannetti, L. Zambreanu, R. G. Wise, T. J. Buchanan, J. P. Huggins, T. S. Smart, W. Vennart, and I. Tracey. Pharmacological modulation of pain-related brain activity during normal and central sensitization states in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 102(50):18195–18200, 2005.

[62] X. Li, C. C. Teneback, Z. Nahas, F. A. Kozel, C. Large, J. Cohn, D. E. Bohning, and M. S. George. Interleaved transcranial magnetic stimulation/functional MRI confirms that lamotrigine inhibits cortical excitability in healthy young men. *Neuropsychopharmacology*, 29(7):1395–407, 2004.

[63] Z. Munoz-Torres, J. L. Armony, D. Trejo-Martinez, R. Conde, and M. Corsi-Cabrera. Behavioural and neural effects of diazepam on a rule-guided response selection task. *Neurosci Res*, 70(3):260–8, 2011.

[64] M. P. Paulus, J. S. Feinstein, G. Castillo, A. N. Simmons, and M. B. Stein. Dose-dependent decrease of activation in bilateral amygdala and insula by lorazepam during emotion processing. *Archives of General Psychiatry*, 62(3):282–288, 2005.

[65] M. Ragnehed, I. Håkansson, M. Nilsson, P. Lundberg, B. Söderfeldt, and M. Engström. Influence of diazepam on clinically designed fMRI. *The Journal of neuropsychiatry and clinical neurosciences*, 19(2):164–172, 2007.

[66] H. Jokeit, M. Okujava, and F. G. Woermann. Carbamazepine reduces memory induced activation of mesial temporal lobe structures: a pharmacological fMRI-study. *BMC Neurol*, 1:6, 2001.

[67] X. Li, C. H. Large, R. Ricci, J. J. Taylor, Z. Nahas, D. E. Bohning, P. Morgan, and M. S. George. Using interleaved transcranial magnetic stimulation/functional magnetic resonance imaging (fMRI) and dynamic causal modeling to understand the discrete circuit specific changes of medications: lamotrigine and valproic acid changes in motor or prefrontal effective connectivity. *Psychiatry Res*, 194(2):141–8, 2011.

[68] X. Li, R. Ricci, C. H. Large, B. Anderson, Z. Nahas, D. E. Bohning, and M. S. George. Interleaved transcranial magnetic stimulation and fMRI suggests that lamotrigine and valproic acid

have different effects on corticolimbic activity. *Psychopharmacology (Berl)*, 209(3):233–44, 2010.

[69] E. C. Bell, M. C. Willson, A. H. Wilman, S. Dave, and P. H. Silverstone. Differential effects of chronic lithium and valproate on brain activation in healthy volunteers. *Hum Psychopharmacol*, 20(6):415–24, 2005.

[70] R. L. Aupperle, L. Ravindran, D. Tankersley, T. Flagan, N. R. Stein, A. N. Simmons, M. B. Stein, and M. P. Paulus. Pregabalin influences insula and amygdala activation during anticipation of emotional images. *Neuropsychopharmacology*, 36(7):1466–77, 2011.

[71] E. A. Thomas and S. Petrou. Network-specific mechanisms may explain the paradoxical effects of carbamazepine and phenytoin. *Epilepsia*, 54(7):1195–202, 2013.

[72] Z. Haneef, H. S. Levin, and S. Chiang. Brain graph topology changes associated with anti-epileptic drug use. *Brain Connect*, 5(5):284–91, 2015.

[73] J. F. Jansen, A. P. Aldenkamp, H. J. Marian Majoie, R. P. Reijs, M. C. de Krom, P. A. Hofman, M. Eline Kooi, K. Nicolay, and W. H. Backes. Functional MRI reveals declined prefrontal cortex activation in patients with epilepsy on topiramate therapy. *Epilepsy Behav*, 9(1):181–5, 2006.

[74] A. De Ciantis, M. Muti, C. Piccolini, M. Principi, A. Di Renzo, R. De Ciantis, D. Frondizi, G. Iannone, P. Ottaviano, and M. Piccirilli. A functional MRI study of language disturbances in subjects with migraine headache during treatment with topiramate. *Neurol Sci*, 29 Suppl 1: S141–3, 2008.

[75] J. P. Szaflarski and J. B. Allendorfer. Topiramate and its effect on fMRI of language in patients with right or left temporal lobe epilepsy. *Epilepsy Behav*, 24(1):74–80, 2012.

[76] C. L. Yasuda, M. Centeno, C. Vollmar, J. Stretton, M. Symms, F. Cendes, M. A. Mehta, P. Thompson, J. S. Duncan, and M. J. Koepp. The effect of topiramate on cognitive fMRI. *Epilepsy Res*, 105(1-2):250–5, 2013.

[77] B. Wandschneider, J. Stretton, M. Sidhu, M. Centeno, L. R. Kozák, M. Symms, P. J. Thompson, J. S. Duncan, and M. J. Koepp. Levetiracetam reduces abnormal network activations in temporal lobe epilepsy. *Neurology*, 83(17):1508–1512, 2014.

[78] B. P. Kay, M. W. DiFrancesco, M. D. Privitera, J. Gotman, S. K. Holland, and J. P. Szaflarski. Reduced default mode network connectivity in treatment-resistant idiopathic generalized epilepsy. *Epilepsia*, 54(3):461–70, 2013.

[79] N. W. Duncan, C. Wiebking, and G. Northoff. Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans – a review of multimodal imaging studies. *Neurosci Biobehav Rev*, 47C:36–52, 2014.

[80] C. J. Stagg, S. Bestmann, A. O. Constantinescu, L. M. Moreno, C. Allman, R. Mekle, M. Woolrich, J. Near, H. Johansen-Berg, and J. C. Rothwell. Relationship between physiological measures of excitability and levels of glutamate and GABA in the human motor cortex. *J Physiol*, 589(Pt 23): 5845–55, 2011.

[81] J. A. Detre, H. Rao, D. J. Wang, Y. F. Chen, and Z. Wang. Applications of arterial spin labeled MRI in the brain. *J Magn Reson Imaging*, 35(5):1026–37, 2012.

[82] P. Beleza. Refractory epilepsy: a clinically oriented review. *Eur Neurol*, 62(2):65–71, 2009.

[83] Z. J. Wang, C. Bergqvist, J. V. Hunter, D. Jin, D. J. Wang, S. Wehrli, and R. A. Zimmerman. In vivo measurement of brain metabolites using two-dimensional double-quantum MR spectroscopy–exploration of GABA levels in a ketogenic diet. *Magn Reson Med*, 49(4):615–9, 2003.

[84] J. W. Pan, R. B. Duckrow, J. Gerrard, C. Ong, L. J. Hirsch, Jr. Resor, S. R., Y. Zhang, O. Petroff, S. Spencer, H. P. Hetherington, and D. D. Spencer. 7T MR spectroscopic imaging in the localization of surgical epilepsy. *Epilepsia*, 54(9):1668–78, 2013.

[85] D. L. Rothman, H. M. De Feyter, R. A. de Graaf, G. F. Mason, and K. L. Behar. 13C MRS studies of neuroenergetics and neurotransmitter cycling in humans. *NMR Biomed*, 24(8):943–57, 2011.

[86] E. A. van Vliet, W. M. Otte, J. A. Gorter, R. M. Dijkhuizen, and W. J. Wadman. Longitudinal assessment of blood-brain barrier leakage during epileptogenesis in rats. a quantitative MRI study. *Neurobiol Dis*, 63:74–84, 2014.

[87] M. P. van den Heuvel, C. J. Stam, R. S. Kahn, and H. E. Hulshoff Pol. Efficiency of functional brain networks and intellectual performance. *J Neurosci*, 29(23):7619–24, 2009.

Chapter 3

Glutamate concentrations vary with antiepileptic drug use and mental slowing

T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, R. H. C. Lazeron, N. A. J. Puts, R. A. E. Edden, P. A. M. Hofman, A. J. A. de Louw, W. H. Backes, J. F. A. Jansen, *Epilepsy&Behavior 2016; 64: 200–205*,
DOI: 10.1007/s10439-014-1198-y

Abstract

Objective: Although antiepileptic drugs (AEDs) are effective in suppressing epileptic seizures, they also induce (cognitive) side effects, with mental slowing as a general effect. This study aimed to assess whether concentrations of the MR detectable neurotransmitters glutamate and GABA are associated with mental slowing in patients with epilepsy taking AEDs.

Methods: Cross-sectional data were collected from 55 patients with localization-related epilepsy using a variety of AEDs from three risk categories, i.e. AEDs with low, intermediate, and high risks of developing cognitive problems. Patients underwent 3T MR spectroscopy, including a PRESS (n=55) and MEGA-PRESS (n=43) sequence, to estimate occipital glutamate and GABA concentrations, respectively. The association was calculated between neurotransmitter concentrations and central information processing speed, which was measured using the Computerized Visual Searching Task (CVST) and compared between the different risk categories.

Results: Combining all groups, patients with lower processing speeds had lower glutamate concentrations. Patients in the high-risk category had a lower glutamate concentration and lower processing speed compared with patients taking low-risk AEDs. Patients taking intermediate-risk AEDs also had a lower glutamate concentration compared with patients taking low-risk AEDs, but processing speed did not differ significantly between those groups. No associations were found between the GABA concentration and risk category or processing speed.

Conclusion: For the first time a relation is shown between glutamate concentration and both mental slowing and AED use. It is suggested that the reduced excitatory action, reflected by lowered glutamate concentrations, may have contributed to the slowing of information processing in patients using AEDs with higher risks of cognitive side effects.

Introduction

Although antiepileptic drugs (AEDs) are effective in suppressing epileptic seizures, they may also induce side effects. These side effects can strongly affect the quality of life of patients, with slowing of central information processing speed as the dominant cognitive effect of most AEDs and also the first sign of cognitive adverse effects [1, 2]. Cognitive side effects are commonly seen among the different AED regimes, but the occurrence and severity vary between different AEDs. The newer AEDs lamotrigine and levetiracetam are suggested to have no adverse, and maybe even beneficial cognitive effects, while topiramate is known for its deleterious cognitive effects. Other AEDs, such as valproate or carbamazepine, are associated with milder cognitive effects [1, 3, 4].

AEDs aim to control epileptic seizures via a number of distinct mechanisms of action, which can be subdivided in suppression of the excitatory mechanisms or enhancement of inhibitory mechanisms [5]. Although cognitive side effects are likely to result from the anticonvulsant activity of the AEDs, these effects cannot be linked to any particular mechanism of action, and other mechanisms might be involved as well [6]. It has been hypothesized that especially AEDs with mechanisms acting on the γ-aminobutyric acid (GABA) system cause cognitive side effects, but similar side effects are also induced by AEDs with other mechanisms of action [7]. Furthermore, it is currently not possible to predict which patients will suffer from these side effects and who will not. However, compliance to AED therapy relies on efficacy as well as tolerance to side effects.

In vivo measurements of the main inhibitory and excitatory neurotransmitters GABA and glutamate can be provided by proton magnetic resonance spectroscopy (^{1}H-MRS). In healthy individuals, higher GABA concentrations and lower glutamate concentrations have been associated with better cognitive performance [8–10]. Previous studies also showed that AED treatment can be associated with altered neurotransmitter concentrations [11]. Although several studies have been performed to associate GABA and glutamate concentrations with seizure control [12–14], to our knowledge the association with cognitive side effects has not been investigated yet. The aim of this cross-sectional study was to assess whether GABA and glutamate concentrations can be linked to cognitive functioning, in terms of decreased processing speed, in patients with epilepsy on long-term AED treatment.

Methods

Patients

Patients with localization-related epilepsy, recruited from our tertiary epilepsy referral center, were included in this study. Inclusion criteria were an age between 18 and 70 years and no contraindications for MRI (metal implants, claustrophobia, or pregnancy). This study was approved by the local Medical Ethical Committee and written informed consent was obtained from all patients before the examination.

To obtain a variation in information processing speed, three groups of patients using different AEDs were included. The groups were defined according to Samarasekera et al. [15], based on the known risk of developing cognitive side effects: a low-risk category (levetiracetam and lamotrigine), an intermediate-risk category (valproate, carbamazepine, oxcarbazepine and phenytoin), and a high-risk category (topiramate). Both patients on mono- and polytherapy were included, but patients took maximal two different AEDs. Patients on polytherapy were classified according to the AED in the highest risk category.

Neuropsychological investigation

Information processing speed was used as a measure for cognitive side effects, as slowing of central information processing speed is the most common side effect of AEDs [2]. For this, the Computerized Visual Searching Task (CVST) was used [16]. In this task, a centered grid pattern has to be compared with 24 surrounding grid patterns. Participants have to find the grid pattern identical to the centered pattern. The score is the average time needed to complete this task. Additionally, as global cognitive abilities are assumed to be unaffected by AEDs [2], the Raven Standard Progressive Matrices was performed to correct for possible variation in cognitive abilities between the patients [17]. This is a non-verbal reasoning test, in which participants have to identify the figure that is required to fulfill a series of eight other figures.

Data acquisition

MR data were acquired on a 3.0T MR scanner equipped with an 8-channel head coil (Philips Achieva, Philips Medical Systems, Best, the Netherlands). Glutamate concentrations were measured using a PRESS sequence (TE/TR: 35/2000 ms, 128 averages, VAPOR water suppression). GABA-edited MR spectra were acquired using a MEGA-PRESS sequence (TE/TR 68/2000 ms, 320 averages, with editing pulses at 1.9 (ON) and 7.46 ppm (OFF) interleaved in 40 blocks, MOIST water

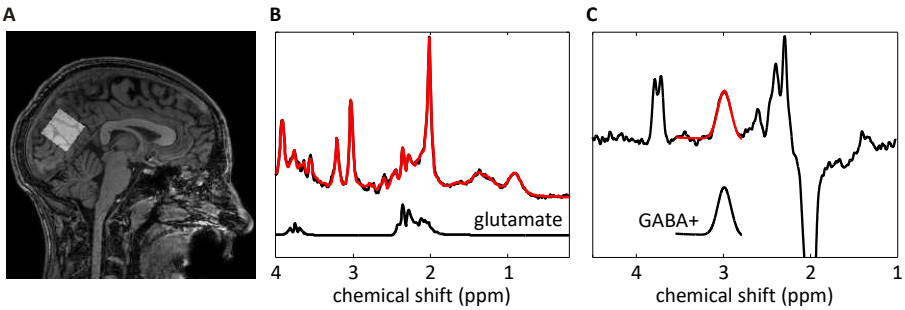

Figure 3.1. Example of the voxel placement (A), a PRESS spectrum with the LCModel fit (in red), which fits a linear combination of metabolite spectra (B), and a MEGA-PRESS spectrum with the GANNET fit (in red), which only fits the GABA+ peak (GABA + co-edited macromolecules) (C). Individual glutamate or GABA fits are displayed in the figures as well.

suppression). Both spectra were acquired from the same 3x3x3 cm^3 voxel located around the parieto-occipital sulcus (Figure 3.1). This location has an optimal signal-to-noise ratio and is commonly selected in MRS studies [18]. To estimate the water signal, separate scans without water suppression were made directly after the PRESS and MEGA-PRESS scans (with TE/TR 35/2000 ms and 128 averages or TE/TR 68/2000 ms and 8 averages, respectively). Additionally, a T1-weighted scan was made to determine the voxel composition (voxel size 1x1x1 mm^3, flip angle 8°, 3D fast spoiled gradient echo sequence, TE/TI/TR 4.8/1022/8.3 ms, 180 slices).

Data analysis

PRESS spectra were analyzed using LCModel (version 6.3-1L). LCModel fits the spectrum with a linear combination of individual metabolite spectra [19]. A standard basis set with sixteen different simulated metabolite spectra was used in this analysis and spectra were analyzed within the resonance frequency range from 0.2 to 4.0 ppm. In addition to glutamate, tNAA (N-acetyl aspartate + N-acetylaspartylglutamate), tCho (phosphorylcholine + glycerophosphorylcholine), and tCr (creatine + phosphocreatine) concentration estimates were collected for further analyses.

Gannet (version 2.0) was used for the preprocessing and quantification of the GABA+ concentration (i.e. GABA and co-edited macromolecules) [20]. The GABA peak is fitted to a Gaussian model curve. Gannet is designed for GABA+ quantification but also enables Glx quantification from MEGA-PRESS spectra [21]. Gannet has the advantage that it also includes frequency and phase corrections [22,

23]. MEGA-PRESS scans are more vulnerable for field drifts and movement artifacts than typical PRESS scans because of the addition of an editing pulse and subtraction of ON and OFF scans to obtain difference spectra, potentially leading to subtraction artifacts. Frequency correction can reduce quantification errors [22]. All spectra were visually inspected on subtraction artifacts and adequate noise levels.

Repeatability of these methods was tested in five healthy volunteers (age 29±4 year, four male), who underwent two PRESS and MEGA-PRESS scans immediately after each other. The results showed a coefficient of variation of 2.9%, 5.2% and 8.1% for glutamate (using PRESS/LCModel), Glx (using MEGA-PRESS/Gannet) and GABA+, respectively. Because of the better coefficient of variation of glutamate estimations with PRESS than Glx estimations with MEGA-PRESS, and because of a moderate concordance between these measurements in the included patients (Pearson correlation coefficient=0.31, p=0.046), only glutamate measurements with PRESS were considered in this study.

All concentrations are reported relative to the unsuppressed water signal from the same volume. FMRIB's Automated Segmentation Tool (FAST), part of FSL (version 5.0.1), was applied to determine the voxel composition in terms of gray matter, white matter, and cerebral spinal fluid (CSF) content [24, 25]. Assuming that the neurometabolites are only present in the gray and white matter, the concentrations relative to the water signal were corrected for the CSF content of the voxel. Therefore, the neurometabolite concentrations were divided by the sum of the gray and white matter fractions.

Statistical analysis

Associations between the neurometabolite concentrations (i.e. glutamate, GABA+, tNAA, tCho, and tCr) and CVST were tested with linear regression analysis, with CVST as dependent variable and the concentrations as independent variables. Separate analyses were performed for each neurometabolite. Besides the neurometabolite concentration, age and the percentage correct answers in the Raven test (as a measure for intelligence) were added to these analyses as independent variables, as the information processing speed usually correlates with age and cognitive abilities.

To test whether the neurometabolite concentrations varied with risk degree an ANCOVA (analysis of covariance) test was applied, with the neurometabolite concentrations as outcome variable and the risk categories as fixed factors. Covariates in the analyses were age, gender, and the gray matter fraction in the voxel (gray matter fraction divided by the sum of the white and gray matter fractions). In

case of significant group effects, post hoc tests (Students t-tests) were applied to test for individual group differences.

To check for possible confounding effects, the analyses were repeated with drug load (defined as the ratio of the prescribed daily dose to the defined daily dose [26]), having symptomatic epilepsy or epilepsy severity added as additional covariate. Epilepsy severity was defined by a composed score ranging from 0 to 7 based on seizure type (tonic-clonic:1, other:0), previous occurrence of status epilepticus (yes:1, no:0), seizure-related injury (yes:1, no:0), and seizure frequency (seizure free:0, yearly:1, monthly:2, weekly:3, daily:4). In all analyses, p-values<0.05 were considered significant.

Results

Patient characteristics

Fifty-eight patients were included in this study. Three of these patients did not finish the MRI examination because of claustrophobia, resulting in suitable data of 55 patients for further analysis. Seventeen of the 55 patients had symptomatic epilepsy. MRI lesions included cerebral atrophy (6), cortical dysplasias (5), infarctions (3), malformations (1), tumors (1), and cysts (1). The remaining 38 patients had non-symptomatic epilepsy.

The low- and intermediate-risk groups differed significantly in age and drug load (Table 3.1). Also the number of patients taking polytherapy was significantly higher in the intermediate- and high-risk categories than in the low-risk category. Patients in the different risk categories did not differ significantly in educational level, seizure frequency, epilepsy severity score, or years since epilepsy onset.

The CVST reaction time ranged from 7.3 to 30.8 s (mean±sd in a healthy adult population: 10.3±4.1 s [28]). Both the patients taking intermediate-risk and high-risk AEDs had a significantly lower processing speed compared with patients taking low-risk AEDs (p=0.003 and p=0.042, respectively, Table 3.1). When age, gender, and the percentage correct answers in the Raven test were added as covariates to this analysis (ANCOVA), there was a significant effect of risk category (p=0.009). Post hoc tests revealed a significantly longer CVST reaction time in the intermediate- (p=0.035, adjusted mean difference: 3.5 s), and in the high-risk categories (p=0.004, adjusted mean difference: 7.8 s), compared with patients taking low-risk AEDs. The CVST reaction time did not differ significantly between the intermediate- and high-risk groups. All participants had a Raven score above the 5th percentile of a healthy, age-matched adult population, and the Raven score was not significantly different between the groups.

Table 3.1. Patient characteristics for the three risk categories[a]. Results are displayed for the participants included in the PRESS analysis.

	Low-risk (n=16)	Intermediate-risk (n=34)	High-risk (n=5)
General			
Male/female	5/11 (31/69%)	16/18 (47/53%)	0/5
Age (years)[b]	39.5±13.4	50.7±12.5*	42.4±15.8
Educational level[c]	5 (range 2-6)	5 (range 2-7)	5 (range 4-6)
Epilepsy-related			
Symptomatic/non-symptomatic epilepsy	2/14 (13/88%)	15/19 (44/56%)	0/5
Seizure frequency			
Weekly	0	1 (3%)	0
Monthly	4 (25%)	3 (9%)	0
Yearly	2 (13%)	6 (18%)	2 (40%)
Seizure free	10 (63%)	24 (71%)	3 (60%)
Years since epilepsy onset[b]	22.7±11.7	30.4±13.4	26.8±23.3
Epilepsy severite score[b]	1.4±0.8	1.2±1.0	1.0±0.7
AED-related			
Mono-/polytherapy	16/0	8/26 (24/77%)*	3/2 (60/40%)†
Medication type			
CBZ	0	17 (50%)	1 (20%)
LEV	7 (44%)	6 (18%)	0
LTG	9 (56%)	10 (29%)	1 (20%)
OXC	0	4 (12%)	0
PHT	0	16 (47%)	0
TPM	0	0	5 (100%)
VPA	0	7 (21%)	1 (20%)
Drug load[b,d]	1.3±0.6	1.8±0.7*	1.2±1.0
Neuropsychological results			
CVST reaction time[b]	11.5±2.9	15.7±6.4*	20.2±6.7†
Raven (% correct answers)[b]	71.7±10.3%	73.2±10.1%	71.7±3.1%

Differences between the risk groups were tested using a Fisher's exact test (gender, symptomatic epilepsy, mono/polytherapy), a Mann-Whitney test (educational level, seizure frequency, epilepsy severity score), or a student's t-test (all remaining variables). *indicates significant differences between the low- and intermediate-risk category ($p<0.05$); †indicates differences between the low- and high-risk categories ($p<0.05$).

[a]Low-risk: lamotrigine (LTG), levetiracetam (LEV); Intermediate-risk: valproate (VPA), carbamazepine (CBZ), oxcarbazepine (OXC) and phenytoin (PHT); High-risk: topiramate (TPM)

[b]mean ± standard deviation

[c]Median (range). Scores are according to Verhage [27], range 1 (did not finish primary school) to 7 (Master's degree)

[d]The drug load is defined as the ratio of the prescribed daily dose to the defined daily dose [28]

Spectroscopy results

The quality of the PRESS spectra was adequate in all patients. Visual inspection did not reveal spectra of insufficient quality, all spectra had a signal-to-noise ratio above 20, and the Cramė r-Rao lower bounds (CRLB) of glutamate were below 10%. Forty-three MEGA-PRESS spectra were included in the statistical analysis (15 from the low-risk group, 26 from the intermediate-risk group, and 2 from the high-risk group). In four patients, no MEGA-PRESS data were available because of acquisition problems, and another eight MEGA-PRESS scans were excluded because of insufficient quality of the spectrum. The error of the GABA fit was below 15% in the remaining spectra, while the mean absolute drift of the water peak between two subsequent blocks was 0.009±0.004 ppm.

Across all participants, the glutamate and GABA concentrations were 9.3±0.8 i.u. (institutional units) and 1.7±0.4 i.u., respectively (mean±sd). tNAA, tCr, and tCho concentrations were 9.0±0.5 i.u., 6.8±0.6 i.u., and 1.2±0.2 i.u., respectively.

The CVST reaction time was significantly associated with glutamate concentration (β=-3.1, p=0.001, with correction for age and global intelligence), indicating that patients with a lower processing speed had a lower glutamate concentration (Figure 3.2). The CVST reaction time was not significantly associated with the GABA (p=0.45), tNAA (p=0.99), tCr (p=0.82), or tCho (p=0.13) concentrations.

The glutamate and GABA concentrations are illustrated for the different groups in Figure 3.3. A significant effect of cognitive risk category on the glutamate concentration was observed (p=0.028, ANCOVA, with age, gender, and gray matter fraction as covariates). Post hoc tests showed a significantly lower glutamate concentration in the intermediate-risk category than in the low-risk category (p=0.021, adjusted mean difference 0.49) and in the high- compared with the low-risk category (p=0.032, adjusted mean difference 0.72). The intermediate- and high-risk categories did not differ significantly in the glutamate concentrations (p=0.47). Risk category did not have a significant effect on the GABA (p=0.83), tNAA (p=0.47), tCho (p=0.085), or tCr (p=0.17) concentration.

The additional analyses, with epilepsy severity score, drug load, or having symptomatic epilepsy added as covariates, showed comparable results as the analyses without these additional covariates (<10% change in effect size). Furthermore, epilepsy severity, drug load, or having symptomatic epilepsy was not a significant covariate in any of the analyses.

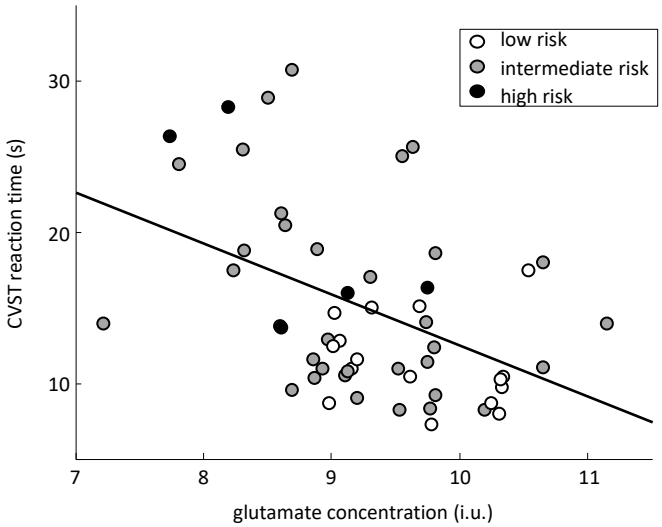

Figure 3.2. Association of the glutamate concentration and CVST reaction time. Patients with lower glutamate concentrations, had a longer CVST reaction time, i.e. a lower processing speed, than patients with higher glutamate concentrations. The depicted line represents the uncorrected linear regression estimate to guide the eye. This association remained significant after correction for age and global intelligence (β=-3.1, p=0.001). It can be noticed that patients taking low-risk AEDs (open circles), had high glutamate concentrations and low-CVST reaction times, while lower glutamate concentrations and higher CVST reaction times were only measured in patients taking intermediate- (gray circles) or high-risk (black circles) AEDs. CVST: computerized visual searching task

Discussion

This study assessed associations between neurotransmitter concentrations, AED treatment, and cognitive functioning in patients with epilepsy. Lower glutamate concentrations were associated with a lower processing speed, the most common type of drug-induced cognitive impairment. Furthermore, patients taking AEDs from higher-risk categories had lower glutamate concentrations than patients taking AEDs from lower-risk categories. No significant associations were found between the GABA+ concentration and risk category or processing speed.

Glutamate concentrations and cognitive slowing

To our knowledge, this is the first study showing associations between neurotransmitter concentrations and drug-induced cognitive side effects (i.e. mental slowing) in patients with epilepsy. Studies in other diseases did also show associations between the glutamate concentration and cognitive functioning, but the variety of the

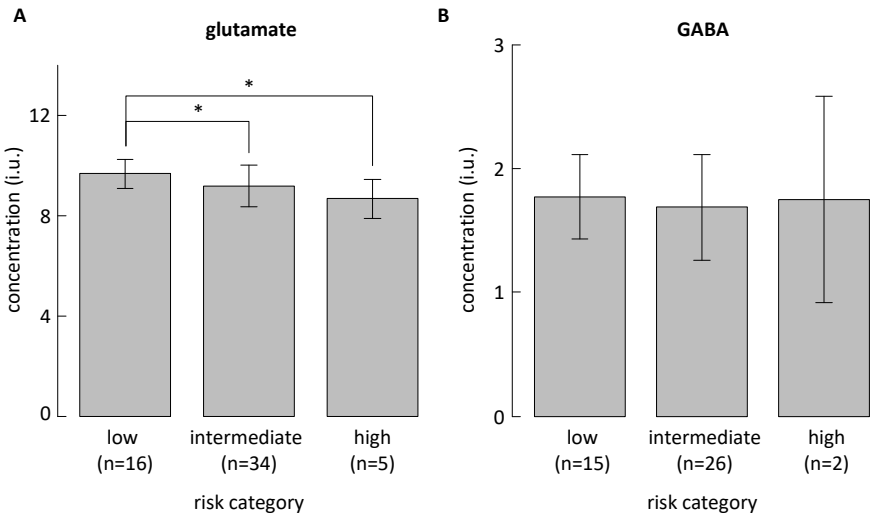

Figure 3.3. Glutamate (A) and GABA (B) concentrations for the three risk groups. Concentrations are relative to the water concentration, corrected for the cerebral spinal fluid content of the voxel, and displayed in institutional units. Low-risk category: lamotrigine or levetiracetam; Intermediate-risk category: carbamazepine, oxcarbazepine, phenytoin or valproate; High-risk category: topiramate. Standard deviations are displayed with error bars. *indicate significant differences ($p<0.05$), when adjusted for age, gender, and gray matter fraction.

applied cognitive tests is large, and both positive and negative associations have been found [29]. Different explanations are proposed for these associations. For instance, it is hypothesized that neurotoxic effects of high glutamate concentrations affect cognitive functioning [30], that a higher GABA/glutamate concentration improves decision-making [8], or that glutamate concentration acts as a marker for neuronal integrity [31]. Precise associations between glutamate and cognitive function and the underlying mechanisms may depend on the specific disease and cognitive functions being studied.

The glutamate concentration measured by MRS is a combination of glutamate functioning as neurotransmitter and glutamate stored in synaptic vesicles and metabolic pools. Although the precise mechanisms are unknown, the glutamate concentration appears to be roughly linearly related to excitatory activity, possibly because of its involvement in the glucose metabolism in the brain [32, 33]. As AEDs generally tend to suppress (abnormal) brain activity [5], it seems likely that the lower glutamate concentrations in this study reflect more suppressed brain activity, which may be associated with a slowing of information processing at the downside of this effect.

AED use and glutamate concentrations

Although differences in glutamate concentrations were observed between the different risk categories, none of the known mechanisms of action of AEDs are likely to affect the glutamate concentration directly [5]. However, it is possible that modulation of the sodium channels, seen in PHT, CBZ, OXC, TPM, and VPA, which blocks high-frequency repetitive action potentials, indirectly decreases the glutamate release. Three previous longitudinal studies showed no changes in the Glx concentration (glutamate and its precursor glutamine combined) after VPA use, or glutamate concentrations after TPM or LEV use [34–36], AEDs which were also used in the current study. Taken together, the current study does not provide evidence that AEDs from different risk categories alter glutamate concentrations directly, but the results do suggest a link between glutamate concentration and mental slowing due to AED use.

GABA

In this study, no associations were found between the GABA concentration and information processing speed or cognitive risk category, in contrast to the hypothesized involvement of GABAergic mechanisms in cognitive side effects of AEDs [7, 37]. Existing associations might have been undetected in this study because of the small group size. Because of exclusion of MEGA-PRESS spectra, this group size was smaller compared with the other results (n=43 versus n=55). Also confounding effects of the different AEDs might have had different effects on the GABA concentration. For instance, both TPM and LTG are suggested to increase the GABA concentration, but a higher increase was reported with TPM use than with LTG use [38]. Using the current clinical study design, it is not feasible to assess distinct effects of the different AEDs, because of the many different combinations of AEDs which were being used by the included patients. However, it cannot be excluded that for individual AEDs, also GABA concentrations are associated with cognitive functioning.

Other neurometabolites

tNaa, tCho, and tCr concentrations were not significantly associated with CVST reaction time nor risk group in this study, while previous studies did show associations with for example NAA and information processing speed [39] or executive functioning [40] in healthy elderly populations. Age-specific mechanisms may underlie these associations, which cannot be generalized to other study populations. For instance, NAA is considered a marker for neuronal integrity and is often asso-

ciated with cognitive functioning. However, cognitive side effects of AED use are reversible and, therefore, not likely to be accompanied by neuronal cell damage as might be the case in aging. Previous studies have not reported associations with tNAA, tCho, or tCr and AED use [11].

Study considerations

This study was performed in patients with epilepsy on long-term AED treatment, which is most relevant for clinical practice. However, inherent to these studies is the heterogeneity of the study population and the different combinations of AEDs that were taken. An important possible confounder in this study is whether patients were on mono- or polytherapy, which largely coincides with taking AEDs from the low- or intermediate-risk category. Importantly, polytherapy itself is already associated with a higher risk of cognitive side effects [41]. It can furthermore not be excluded that both glutamate concentrations and processing speed were affected by epilepsy characteristics or underlying causes rather than AED use. Future studies are needed to distinguish these factors, and to clarify the precise, causal relationship between neurotransmitter concentrations, AED use, and cognitive side effects.

Because of its negative cognitive side effects, topiramate is not commonly prescribed in our epilepsy center. This resulted in a limited number of patients taking high-risk AEDs in this study, but the results of this group are in line with the results from the intermediate-risk category. The lack of significant differences between the intermediate- and high-risk categories might therefore be due to the small group size of the high-risk category.

The MRS measurements used in this study showed good repeatability. However , the low-concordance between glutamate estimations from PRESS and Glx from MEGA-PRESS is striking. A possible explanation is the presence of macromolecules in one of these spectra, but future studies are prompted to investigate this topic. A final consideration regarding MRS measurements is the voxel location. As in many previous studies, the occipital lobe was chosen because it gives the best signal-to-noise ratio [18, 22]. However, regions important for cognitive processes include the prefrontal cortex and subcortical structures, and not the occipital lobe [42]. Currently, it remains to be determined whether relevant spatial variations exist in the neurotransmitter concentrations in relation to adverse cognitive effects.

Future perspectives

Currently, it is not possible to predict which patients will or will not suffer from these side effects, though this would aid clinical decision making. The results of this study show the potential of glutamate measurements as a candidate biomarker. In order to predict these side effects, it is necessary to first longitudinally assess changes in neurotransmitter concentration, to see whether the differences in glutamate levels possibly precede the cognitive problems or coincide with the cognitive problems.

Conclusion

For the first time a relation is shown between lowered glutamate concentrations and both AED use and mental slowing in patients with epilepsy. This observation hints at a possible contribution to a neurobiological mechanism of mental slowing due to AED use. More knowledge about this relation might help to explain why some patients with epilepsy experience cognitive side effects while the cognitive function of other patients is not affected by AEDs. Future studies with MRS and AEDs are warranted to further elucidate more details of underlying mechanisms.

Acknowledgements

The authors thank R. Berting for his assistance with the image acquisition and M. Geerlings and J. Slenter for continuous hardware and software support.

References

[1] D. M. IJff and A. P. Aldenkamp. Cognitive side-effects of antiepileptic drugs. *Handb Clin Neurol.*, 111:707–718, 2013.

[2] E. Grevers, L. E. Breuer, I. Jff DM, and A. P. Aldenkamp. Mental slowing in relation to epilepsy and antiepileptic medication. *Acta Neurol Scand*, 2015.

[3] C. Luoni, F. Bisulli, M. P. Canevini, G. De Sarro, C. Fattore, C. A. Galimberti, G. Gatti, A. La Neve, G. Muscas, L. M. Specchio, S. Striano, E. Perucca, and Sophie Study Group. Determinants of health-related quality of life in pharmacoresistant epilepsy: results from a large multicenter study of consecutively enrolled patients using validated quantitative assessments. *Epilepsia*, 52 (12):2181–91, 2011.

[4] R. S. Fisher, B. G. Vickrey, P. Gibson, B. Hermann, P. Penovich, A. Scherer, and S. Walker. The impact of epilepsy from the patient's perspective II: views about therapy and health care. *Epilepsy Research*, 41(1):53–62, 2000.

[5] M. A. Rogawski and W. Loscher. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*, 5 (7):553–64, 2004.

[6] S. A. Hamed. The aspects and mechanisms of cognitive alterations in epilepsy: the role of antiepileptic medications. *CNS Neurosci Ther*, 15(2):134–56, 2009.

[7] R. Sankar and G. L. Holmes. Mechanisms of action for the commonly used antiepileptic drugs: Relevance to antiepileptic drug–associated neurobehavioral adverse effects. *Journal of Child Neurology*, 19(1 suppl):S6–S14, 2004.

[8] G. Jocham, L. T. Hunt, J. Near, and T. E. Behrens. A mechanism for value-guided choice based on the excitation-inhibition balance in prefrontal cortex. *Nat Neurosci*, 15(7):960–1, 2012.

[9] K. Sandberg, J. U. Blicher, M. Y. Dong, G. Rees, J. Near, and R. Kanai. Occipital GABA correlates with cognitive failures in daily life. *Neuroimage*, 87:55–60, 2014.

[10] P. Sumner, R. A. Edden, A. Bompas, C. J. Evans, and K. D. Singh. More GABA, less distraction: a neurochemical predictor of motor decision speed. *Nat Neurosci*, 13(7):825–7, 2010.

[11] T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, P. A. Hofman, M. C. Vlooswijk, R. P. Rouhl, A. J. de Louw, W. H. Backes, and J. F. Jansen. Metabolic and functional MR biomarkers of antiepileptic drug effectiveness: A review. *Neurosci Biobehav Rev*, 59:92–9, 2015.

[12] S. G. Mueller, O. M. Weber, C. O. Duc, B. Weber, D. Meier, W. Russ, P. Boesiger, and H. G. Wieser. Effects of vigabatrin on brain GABA+/Cr signals in patients with epilepsy monitored by 1H-NMR-spectroscopy: Responder characteristics. *Epilepsia*, 42(1):29–40, 2001.

[13] S. G. Mueller, O. M. Weber, C. O. Duc, D. Meier, W. Russ, P. Boesiger, and H. G. Wieser. Effects of vigabatrin on brain GABA+/Cr signals in focus distant and focus near brain regions monitored by 1H-NMR spectroscopy. *European Journal of Neurology*, 10(1):45–52, 2003.

[14] O. A. C. Petroff, K. L. Behar, R. H. Mattson, and D. L. Rothman. Human brain γ-aminobutyric acid levels and seizure control following initiation of vigabatrin therapy. *Journal of neurochemistry*, 67(6):2399–2404, 1996.

[15] S. R. Samarasekera, C. Helmstaedter, and M. Reuber. Cognitive impairment in adults with epilepsy: The relationship between subjective and objective assessments of cognition. *Epilepsy Behav*, 52(Pt A):9–13, 2015.

[16] A. P. Aldenkamp, J. Arends, N. M. de la Parra, and E. J. W. Migchelbrink. The cognitive impact of epileptiform EEG discharges and short epileptic seizures: relationship to characteristics of the cognitive tasks. *Epilepsy & Behavior*, 17(2):205–209, 2010.

[17] J. Raven, J. C. Raven, and J. H. Court. Manual for Raven's Progressive Matrices and Vocabulary Scales. Section 3: The Standard Progressive Matrices. *San Antonio, TX: Harcourt Assessment*, 2000.

[18] N. A. Puts and R. A. Edden. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog Nucl Magn Reson Spectrosc*, 60:29–41, 2012.

[19] S. W. Provencher. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, 30(6):672–679, 1993.

[20] R. A. Edden, N. A. Puts, A. D. Harris, P. B. Barker, and C. J. Evans. Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *J Magn Reson Imaging*, 40(6):1445–52, 2014.

[21] R. L. O'Gorman, L. Michels, R. A. Edden, J. B. Murdoch, and E. Martin. In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J Magn Reson Imaging*, 33(5):1262–7, 2011.

[22] C. J. Evans, N. A. Puts, S. E. Robson, F. Boy, D. J. McGonigle, P. Sumner, K. D. Singh, and R. A. Edden. Subtraction artifacts and frequency (mis-)alignment in J-difference GABA editing. *J Magn Reson Imaging*, 38(4):970–5, 2013.

[23] J. Near, R. Edden, C. J. Evans, R. Paquin, A. Harris, and P. Jezzard. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. *Magn Reson Med*, 73(1):44–50, 2015.

[24] Y. Zhang, M. Brady, and S. Smith. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *Medical Imaging, IEEE Transactions on*, 20(1):45–57, 2001.

[25] S. M. Smith, M. Jenkinson, M. W. Woolrich, C. F. Beckmann, T. E. Behrens, H. Johansen-Berg, P. R. Bannister, M. De Luca, I. Drobnjak, D. E. Flitney, R. K. Niazy, J. Saunders, J. Vickers, Y. Zhang, N. De Stefano, J. M. Brady, and P. M. Matthews. Advances in functional and structural

MR image analysis and implementation as FSL. *NeuroImage*, 23 Suppl 1:S208–19, 2004.

[26] M. W. Lammers, Y. A. Hekster, A. Keyser, H. Meinardi, W. O. Renier, and H. van Lier. Monotherapy or polytherapy for epilepsy revisited: a quantitative assessment. *Epilepsia*, 36(5):440–446, 1995.

[27] F. Verhage. *Intelligentie en leeftijd: Onderzoek bij Nederlanders van twaalf tot zevenenzeventig jaar*. Van Gorcum Assen, 1964.

[28] W. Alpherts and A. P. Aldenkamp. FePsy: the iron psyche. *Heemstede: Instituut voor Epilepsiebestrijding*, 1994.

[29] G. Ende. Proton magnetic resonance spectroscopy: Relevance of glutamate and GABA to neuropsychology. *Neuropsychol Rev*, 25(3):315–25, 2015.

[30] I. K. Lyoo, S. J. Yoon, G. Musen, D. C. Simonson, K. Weinger, N. Bolo, C. M. Ryan, J. E. Kim, P. F. Renshaw, and A. M. Jacobson. Altered prefrontal glutamate–glutamine–γ-aminobutyric acid levels and relation to low cognitive performance and depressive symptoms in type 1 diabetes mellitus. *Archives of general psychiatry*, 66(8):878–887, 2009.

[31] P. G. Unschuld, R. A. Edden, A. Carass, X. Liu, M. Shanahan, X. Wang, K. Oishi, J. Brandt, S. S. Bassett, G. W. Redgrave, R. L. Margolis, P. C. van Zijl, P. B. Barker, and C. A. Ross. Brain metabolite alterations and cognitive dysfunction in early huntington's disease. *Mov Disord*, 27(7): 895–902, 2012.

[32] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[33] Jr. Novotny, E. J., R. K. Fulbright, P. L. Pearl, K. M. Gibson, and D. L. Rothman. Magnetic resonance spectroscopy of neurotransmitters in human brain. *Ann Neurol*, 54 Suppl 6:S25–31, 2003.

[34] R. J. Simister, M. A. McLean, G. J. Barker, and J. S. Duncan. The effect of sodium valproate on proton MRS visible neurochemical concentrations. *Epilepsy Res*, 74(2-3):215–9, 2007.

[35] C. M. Moore, M. Wardrop, B. Frederick B. de, and P. F. Renshaw. Topiramate raises anterior cingulate cortex glutamine levels in healthy men; a 4.0T magnetic resonance spectroscopy study. *Psychopharmacology (Berl)*, 188(2):236–43, 2006.

[36] M. H. Pollack, J. E. Jensen, N. M. Simon, R. E. Kaufman, and P. F. Renshaw. High-field MRS study of GABA, glutamate and glutamine in social anxiety disorder: response to treatment with levetiracetam. *Prog Neuropsychopharmacol Biol Psychiatry*, 32(3):739–43, 2008.

[37] M. Mula and M. R. Trimble. Antiepileptic drug-induced cognitive adverse effects. *CNS drugs*, 23 (2):121–137, 2009.

[38] R. Kuzniecky, S. Ho, J. Pan, R. Martin, F. Gilliam, E. Faught, and H. Hetherington. Modulation of cerebral GABA by topiramate, lamotrigine, and gabapentin in healthy adults. *Neurology*, 58 (3):368–372, 2002.

[39] P. Kochunov, T. Coyle, J. Lancaster, D. A. Robin, J. Hardies, V. Kochunov, G. Bartzokis, J. Stanley, D. Royall, A. E. Schlosser, et al. Processing speed is correlated with cerebral health markers in the frontal lobes as quantified by neuroimaging. *NeuroImage*, 49(2):1190–1199, 2010.

[40] R. A. Charlton, D. J. O. McIntyre, F. A. Howe, R. G. Morris, and H. S. Markus. The relationship between white matter brain metabolites and cognition in normal aging: the GENIE study. *Brain research*, 1164:108–116, 2007.

[41] P. Kwan and M. J. Brodie. Neuropsychological effects of epilepsy and antiepileptic drugs. *The Lancet*, 357(9251):216–222, 2001.

[42] R. Cabeza and L. Nyberg. Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of cognitive neuroscience*, 12(1):1–47, 2000.

Chapter 4

Chronic antiepileptic drug use and functional network efficiency

T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, R. H. C. Lazeron, P. A. M. Hofman, A. J. A. de Louw, W. H. Backes, J. F. A. Jansen,
World Journal of Radiology (In press)

Abstract

Objective: To increase our insight in the neuronal mechanisms underlying cognitive side effects of antiepileptic drug (AED) treatment.

Methods: The relation between functional MR-acquired brain network measures, AED use, and cognitive function was investigated. Three groups of patients with epilepsy with a different risk profile for developing cognitive side effects were included: a 'low-risk' category (lamotrigine or levetiracetam, n=16), an 'intermediate-risk' category (carbamazepine, oxcarbazepine, phenytoin, or valproate, n=34) and a 'high-risk' category (topiramate, n=5). Brain connectivity was assessed using resting state functional MRI and graph theoretical network analysis. The Computerized Visual Searching Task was used to measure central information processing speed, a common cognitive side effect of AED treatment.

Results: Central information processing speed was lower in patients taking AEDs from the intermediate- and high-risk categories, compared with patients from the low-risk category. The effect of risk category on global efficiency was significant ($p<0.05$, ANCOVA), with a significantly higher global efficiency for patient from the low-risk category compared with the high-risk category ($p<0.05$, post hoc test). Risk category had no significant effect on the clustering coefficient (ANCOVA, $p>0.2$). Also no significant associations between information processing speed and global efficiency or the clustering coefficient (linear regression analysis, $p>0.15$) were observed.

Conclusion: Only the four patients taking topiramate show aberrant network measures, suggesting that alterations in functional brain network organization may be only subtle and measureable in patients with more severe cognitive side effects.

Introduction

Epilepsy is generally treated with antiepileptic drugs (AEDs). A persistent problem in AED treatment is the occurrence of adverse events among which cognitive side effects are commonly seen [1, 2]. The cognitive side effects account for a high percentage of the disease burden [3] and lead to early drug discontinuation [4]. The prevalence and severity of the cognitive side effects varies among different AEDs. Several AEDs, such as topiramate, are associated with cognitive problems such as language deficit (anomia), while other AEDs such as lamotrigine seem to induce less cognitive side effects or even have activating effects [5]. Despite specific differences, a decreased central information processing speed is commonly observed among the different AEDs to some extent [2].

AEDs control epileptic seizures via several distinct mechanisms, such as enhancement of GABAergic inhibition, reduction of glutamatergic neurotransmission, or modulation of the voltage-gated ion channels [6]. Changes in brain metabolic processes also affect healthy brain activity, and are likely to be responsible for cognitive side effects [1]. Functional magnetic resonance imaging (fMRI) enables assessment of this brain activity, and can be employed to measure combined effects of different mechanism of action of AEDs [7]. Several fMRI studies have shown altered brain activity patterns in healthy participants [8] or patients with epilepsy [9, 10] treated with AEDs. For instance, altered brain activity patterns appeared to be associated with language impairments when taking topiramate [11–13].

Cognitive functions are mediated by the concerted action of multiple and distributed brain regions. These brain regions show correlations of their fMRI time signals, which is commonly interpreted as functional connectivity. Collectively, these functional connections form a brain network, which can be analyzed and characterized using graph theoretical analysis. Brain networks appear to be efficient networks, characterized by a high functional segregation and integration, i.e. different brain regions form densely interconnected groups, enabling specialized information processing, and also rapid communication between distributed brain regions. Several graph measures are available to quantify these characteristics [14].

Cognitive performance has been associated with the efficiency of functional brain networks [15, 16], while impaired functional brain networks have been associated with cognitive decline in epilepsy [17, 18]. Furthermore, associations between drug load, cognition and graph measures were shown in one of these studies, although this was not the main focus of the current study [17]. Another study associated the use of carbamazepine with altered graph measures when compared with other AEDs, but did not investigate the relation with cognitive effects [19]. In

the current study, we aim to test whether chronic use of AEDs, associated with a high risk for cognitive side effects, affects functional resting-state network measures differently than long-term use of AEDs associated with milder cognitive side effects. Furthermore, we will test whether functional resting-state network measures are associated with impaired cognitive functioning.

Methods

Patients

Three groups of patients with epilepsy were compared in this observational, cross-sectional study [20]. These groups were subdivided based on the AEDs that were being used, in accordance to Samarasekera et al. [21]. The first group, the low-risk category, consisted of patients using lamotrigine or levetiracetam. Patients taking carbamazepine, oxcarbazepine, phenytoin, or valproate were included in the intermediate-risk category, while the high-risk category comprised patients taking topiramate. Patients on polytherapy took at most two different AEDs and were categorized according to their AED associated with the greatest cognitive risk. By including patients with AEDs from the three risk groups, a range in slowing of information processing speed is set out for.

All patients were clinically diagnosed with localization-related epilepsy and aged between 18 and 70 years. The patients were recruited from our tertiary epilepsy referral center. Participants not eligible for MRI, because of metal implants, claustrophobia, or pregnancy, were excluded from this study. Furthermore, patients did not experience seizures at least 12 hours prior to MRI. This study was approved by the local Medical Ethical Committee and all participants provided written informed consent.

Neuropsychological investigation

Cognitive functioning was assessed by two neuropsychological tasks. The Computerized Visual Searching Task (CVST) was used to measure visual (complex) information processing speed [22]. Slowing of this central information processing speed is a common side effect of AEDs [2], and therefore the CVST is considered to be sensitive for treatment effects [23]. With the CVST, a centered grid is shown surrounded by 24 other grid patterns. Participants have to find the (only) grid identical to the centered one as fast as possible.

The Raven Standard Progressive Matrices was administered to assess global cognitive performance. This is a non-verbal reasoning test which gives an indication

of fluid intelligence [24]. Previous studies suggested that intelligence stays relatively unaffected by AEDs [25].

Epilepsy severity

As several epilepsy related characteristics might affect functional brain networks [26], a score was composed to account for these effects. This epilepsy severity score was assessed in all patients and compared between the different risk categories. Epilepsy severity was characterized using a summarized score between zero and seven, composed by the sum of subscores for seizure type (tonic-clonic: 1, other: 0), previous occurrence of status epilepticus (yes: 1, no: 0), seizure-related injury (yes: 1, no: 0) and seizure frequency (seizure free: 0, yearly: 1, monthly: 2, weekly: 3, daily: 4).

MRI data acquisition

MRI data were acquired on a 3.0T MRI scanner equipped with an 8-channel head coil (Philips Achieva, Philips Medical Systems, Best, the Netherlands). The scanning protocol included resting-state functional MRI and a T1-weighted scan. Functional MRI data were acquired using whole-brain single-shot multi-slice echo planar imaging (EPI) sequence sensitive to the blood-oxygen-level-dependent (BOLD) effect (195 volumes, 32 slices, in-plane resolution 2x2 mm^2, 4 mm thick slices, repetition time 2000 ms, echo time 35 ms, flip angle: 90°, acquisition time: 7 min). A 3D T1-weighted scan was acquired for anatomic reference (voxel size 1x1x1 mm^3, repetition time 8.3 ms, echo time 4.8 ms, inversion time 1022 ms, 180 slices, flip angle 8°, acquisition time 6 min).

Data preprocessing

Preprocessing of the functional images was performed using SPM8 (Wellcome Department of Cognitive Neurology, London, UK). The functional images were corrected for differences in slice timing and head movement, coregistered to the T1 image and spatially (FWHM 6 mm) and temporally filtered (band pass 0.01–0.1 Hz). The BOLD signal originating from the white matter and ventricles, which is assumed to reflect physiological noise [27], and the six translation and rotation parameters obtained from the motion correction were deregressed from the BOLD signal.

The T1-weighted scan was parcellated into 82 cortical and subcortical brain regions using FreeSurfer v5.1.0 (The General Hospital Corporation, Boston MA, USA). Subsequently, a connectivity matrix was created by calculating the Pear-

son's correlation coefficient between the average (deregressed) BOLD time signal of each combination of two regions. Negative correlations were set to zero. The correlation values were thresholded, based on the average connectivity matrix, to obtain connectivity matrices with only the strongest connections. The number of included connections was varied, with sparsity levels ranging from 0 to 0.9 (0 is fully connected, whereas 1 indicates no connections).

Data analysis

The Brain Connectivity Toolbox [14] was employed to compute graph measures for each individual connectivity matrix. The clustering coefficient and the characteristic path length are commonly used to characterize the functional segregation and integration, respectively. The clustering coefficient quantifies the fraction of a node's neighbor that are also connected to each other. The characteristic path length is defined as the average shortest distance (the inverse correlation coefficient) between all pairs of nodes. As, in sparse networks, a single weak connection can result in a large, or even infinite average path lengths, global efficiency was computed instead of characteristic path length, which avoids this effect by using inverse path lengths [28].

One hundred null models of the connectivity matrices were computed by randomizing the connections of the original matrices, while preserving the degree and weight distribution [29]. The graph measures were divided by the mean global efficiency and clustering coefficient of these null models, providing a normalized global efficiency (Eg) and clustering coefficient (γ).

Statistical analysis

To test whether the clustering coefficient and global efficiency differed between the risk categories, an analysis of covariance (ANCOVA) was applied with the graph measures as outcome, cognitive risk category as fixed factor and age as covariate. Associations with cognition were assessed with linear regression analysis, with CVST time as outcome, and Eg or γ, age, and the percentage corrects answers in the Raven test as independent variables. To assess whether these results were affected by confounders, these analyses were repeated with gender, epilepsy severity score, or drug load (ratio of prescribed daily dose to defined daily dose [30]) added to the regression analyses as additional covariates. All statistical analyses were performed in MATLAB (version R2012b). P-values lower than 0.05 were considered significant.

Results

Patient characteristics

In total, 58 patients were included in this study. Three of these patients did not finish the procedures due to claustrophobia, resulting in 16 patients taking AEDs from the low-risk category, 34 taking AEDs from the intermediate-risk category, and 5 taking high-risk AEDs. The age and drug load were significantly higher in the intermediate-risk category than in the low-risk category (Table 4.1). Also the number of patients on polytherapy was significantly higher in the intermediate-risk category compared with the low-risk category, while the high- and low-risk categories significantly differed in number of patients on polytherapy. The risk categories did not differ in gender distribution, educational level, or epilepsy severity.

Neuropsychological assessment

The results of the CVST and the Raven task are summarized in Table 4.2. The CVST reaction time was slower than the normal range (range: 7.3 to 30.8 s, while the mean±sd was 10.3±4.1 s in normal population [32]). A significant effect of risk category on CVST reaction time was observed, which remained significant when controlling for age, gender, and global cognitive level (p=0.009, ANCOVA). Post hoc tests showed significant differences in CVST between the low- and intermediate-risk category (p=0.035, estimated adjusted mean difference 3.5 s), and between the low- and high-risk category (p=0.004, adjusted mean difference 7.8 s). No significant differences were found between the percentage correct answers Raven scores of the different risk categories.

Network topology

Of the 55 included patients, seven were excluded from further analysis: one patient was excluded because of excessive head motion (maximum head movement of 8.0 mm, while the maximum head movement was below 1.5 mm in all other patients), one because of a deeper large lesion mass, and five patients were excluded because of a failure to automatically parcellate the cortex, due to cortical abnormalities. The analysis was therefore performed on 48 patients: 15 patients taking AEDs from the low-risk category, 29 patients taking AEDs from the intermediate-risk category and 4 patients taking the high-risk medication. The maximum head displacement did not differ between the three risk categories. The functional networks were fully connected and showed small-world characteristics within the sparsity range 0.32–0.66 (which was defined as γ/λ significantly larger than one, with γ the nor-

Table 4.1. Patient characteristics for the three risk categories[a].

	Low-risk (n=16)	Intermediate-risk (n=34)	High-risk (n=5)
General			
Male/female	5/11 (31/69%)	16/18 (47/53%)	0/5 (0/100%)
Age (years)[b]	39.5±13.4	50.7±12.5*	42.4±15.8
Educational level[c]	5 (range 2–6)	5 (range 2–7)	5 (range 4–6)
Epilepsy-related			
Symptomatic/non-symptomatic epilepsy	2/14 (13/88%)	15/19 (44/56%)	0/5
Seizure frequency			
Weekly	0	1 (3%)	0
Monthly	4 (25%)	3 (9%)	0
Yearly	2 (13%)	6 (18%)	2 (40%)
Seizure free	10 (63%)	24 (71%)	3 (60%)
Years since epilepsy onset[b]	22.7±11.7	30.4±13.4	26.8±23.3
Epilepsy severity score[b]	1.4±0.8	1.2±1.0	1.0±0.7
AED-related			
Mono-/polytherapy	16/0	8/26 (24/77%)*	3/2 (60/40%)†
Medication type			
CBZ	0	17 (50%)	1 (20%)
LEV	7 (44%)	6 (18%)	0
LTG	9 (56%)	10 (29%)	1 (20%)
OXC	0	4 (12%)	0
PHT	0	16 (47%)	0
TPM	0	0	5 (100%)
VPA	0	7 (21%)	1 (20%)
Drug load[b,d]	1.3±0.6	1.8±0.7*	1.2±1.0

Differences between the risk groups were tested using a Fisher's exact test (gender, symptomatic epilepsy, number of different AEDs), a Mann-Whitney test (educational level, seizure frequency, epilepsy severity score), or a student's t-test (all remaining variables). *indicates significant differences between the low- and intermediate-risk category ($p<0.05$); †indicates differences between the low- and high-risk category ($p<0.05$).

[a]Low-risk: lamotrigine (LTG), levetiracetam (LEV); Intermediate-risk: valproate (VPA), carbamazepine (CBZ), oxcarbazepine (OXC) and phenytoin (PHT); High-risk: topiramate (TPM)

[b]mean±standard deviation

[c]Median (range). Scores are according to Verhage [31], range 1 (did not finish primary school) to 7 (Master's degree)

[d]The drug load is defined as the ratio of the prescribed daily dose to the defined daily dose [30].

malized clustering coefficient, and λ the normalized characteristic path length). Only the sparsity levels within this range were considered for further analyses. The ANCOVA test revealed significant effects of risk category on Eg at most sparsities within this sparsity range (Figure 4.1). Post hoc tests showed a significantly higher Eg for patients from the low-risk category (n=14) compared with the high-risk category (n=4), and for patients from the intermediate-risk category (n=29)

Table 4.2. Results of the neuropsychological investigation, represented as mean±standard deviation for each risk category.

	Cognitive test results	
	CVST (s)[a]	Raven[b]
Risk category		
Low-risk	11.5±2.9	71.7±10.3%
Intermediate-risk	15.7±6.4	73.2±10.1%
High-risk	20.2±6.7	71.7±3.1%
p-value[c]	0.008	0.85

[a]mean reaction time on the Computerized Visual Searching Task (CVST) [22]
[b]Percentage correct answers on the Raven Standard Progressive Matrices [24]
[c]Tested with ANOVA

compared with the high-risk category (n=4). Eg or γ did not differ significantly between patients from the low- and intermediate-risk categories (p>0.2 at all sparsity levels), and no significant associations were observed between γ or Eg and CVST time (p>0.15 at all sparsity levels). Gender, epilepsy severity score, or drug load were not significantly associated with the γ, Eg, or CVST reaction time, and the results of these adjusted analyses were consistent with the results of the analyses without these additional covariates (<10% change in effect size of the variable of interest).

Discussion

The current study investigated whether patients taking AEDs with a different risk for cognitive side effects have different functional brain topologies. To this end, we included epilepsy patients with chronic AED treatment with different risk profiles, i.e. a low-risk category, intermediate-risk, and high-risk category. Furthermore, we assessed whether cognitive problems, in terms of a decreased central information processing speed, could be associated with the functional brain organization.

A higher global efficiency was shown in patients taking TPM (n=4, the high-risk category), compared with patients taking the low- (n=14) and intermediate-risk AEDs (n=29). The directionality of this difference is strikingly, as this result seems to contradict the cognitive side effects of TPM. The global efficiency is suggested to be particularly important for more complex cognitive tasks, for which different brain areas are involved [33]. The 'better' global efficiency in TPM users might however be interpreted as a compensatory mechanism, or could be explained by a 'survivor effect'. As patients with side effects are more likely to switch to other

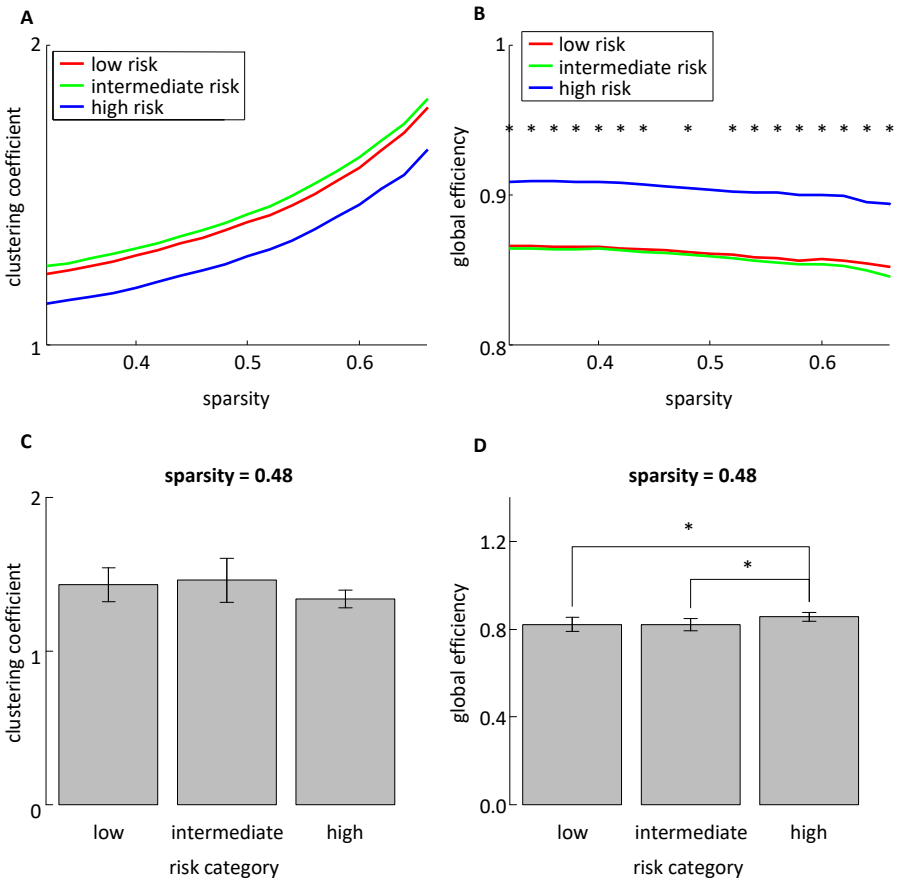

Figure 4.1. Mean clustering coefficient (A, C) and global efficiency (B, D) for each risk category. Both clustering coefficient and global efficiency are normalized, i.e. the measures are divided by the clustering coefficient and global efficiency of random networks. A and B show the graph measures as a function of sparsity, while B and D show the results at a single sparsity level. Error bars show standard deviations, while the asterisks indicate significant differences between the risk categories ($p<0.05$, with age included as covariate).

AEDs, it is likely that these patients are less vulnerable for cognitive problems. The higher global efficiency in the high-risk group might therefore reflect a lower susceptibility for cognitive side effects of these patients [34, 35]. However, these patients did have a lower processing speed compared with the other patients, which argues against this explanation and in favor of a compensatory mechanism.

No differences in graph measures were observed between the patients groups taking AEDs from the low- and from the intermediate-risk category. It is possible that the effects of TPM on brain organization are more pronounced compared with effects of other AEDs, but TPM can also have distinctive effects on brain organization. TPM is suggested to have a unique cognitive profile, with specific effects on verbal fluency. Moreover, it has multiple mechanisms of action, and both these mechanisms and its chemical structure differ from other AEDs [36].

Furthermore, no associations were found between processing speed and graph measures, in contrast to a previous study that showed not only associations between intellectual decline and a lowered clustering coefficient in patients with epilepsy, but also with increasing drug load [17]. The latter suggests that the intellectual decline (which was based on intelligence tests) was a side effect of the AED treatment, but this could also result from differences in epilepsy characteristics. That study included more patients with a high drug load (15% of the patients had a drug load higher than 3) than the current study (no drug loads higher than 3 in the included patients), thus it is possible that the effects on graph measures are only measureable in patients with higher drug loads or AEDs with high risks on cognitive complaints.

The measured information processing speed covered the whole range from normal to a clearly affected processing speed, and patients taking AEDs known to induce cognitive side effects, showed lower processing speeds than patients with lower risk AEDs. These results could therefore not explain the lack of associations between graph measures and information processing speed, or the lack of differences in graph measures between the low- and intermediate-risk category. Also no trends were shown, while the total number of participants (48), and the number of patients in the low- (16) and intermediate-risk categories (34) were relatively large, making it unlikely that this lack of findings were due to limited power.

All included patients in the current study were diagnosed with localization-related epilepsy. Epilepsy is associated with a decreased global efficiency and increased clustering coefficient, although some studies showed a decreased clustering coefficient in patients with epilepsy [37]. It is therefore plausible that the functional brain networks of all three groups of patients in this study were already altered compared with healthy participants, irrespective of AED treatment.

This study has several limitations. Although we tried to include comparable patient groups, the risk categories differed in age and drug load, suggesting that our

study population is biased. Therefore, the analyses were corrected for these characteristics by including age and drug load as covariates. Besides these characteristics, also other factors could have confounded our results, such as the location of the epileptic focus or effects of AEDs on the neurovascular coupling, which should be assessed in separate studies [38]. Finally, no information is available about changes over time and causality due to the cross-sectional design.

Conclusion

No differences in functional network graph measures could be detected between patients with epilepsy after chronic use of AEDs with a different risks on cognitive side effects. Only the four patients taking TPM, which has a high risk for developing cognitive side effects, showed a more efficient brain network topology, which might be a compensatory mechanism. Also no associations were found between the graph measures and the measured cognitive impairments, specifically slowing of central information processing. Alterations in functional brain network organization may be only subtle and measureable in patients with more severe cognitive side effects.

Acknowledgements

The authors thank R. Berting for his assistance with the image acquisition and M. Geerlings and J. Slenter for continuous hardware and software support.

References

[1] P. Perucca and F. G. Gilliam. Adverse effects of antiepileptic drugs. *The Lancet Neurology*, 11 (9):792–802, 2012.

[2] D. M. IJff and A. P. Aldenkamp. Cognitive side-effects of antiepileptic drugs. *Handb Clin Neurol.*, 111:707–718, 2013.

[3] C. Helmstaedter, A. P. Aldenkamp, G. A. Baker, A. Mazarati, P. Ryvlin, and R. Sankar. Disentangling the relationship between epilepsy and its behavioral comorbidities – the need for prospective studies in new-onset epilepsies. *Epilepsy Behav*, 31:43–7, 2014.

[4] H. P. Bootsma, L. Ricker, Y. A. Hekster, J. Hulsman, D. Lambrechts, M. Majoie, A. Schellekens, M. de Krom, and A. P. Aldenkamp. The impact of side effects on long-term retention in three new antiepileptic drugs. *Seizure*, 18(5):327–331, 2009.

[5] P. Kwan and M. J. Brodie. Neuropsychological effects of epilepsy and antiepileptic drugs. *The Lancet*, 357(9251):216–222, 2001.

[6] M. A. Rogawski and W. Loscher. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*, 5 (7):553–64, 2004.

[7] T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, P. A. Hofman, M. C. Vlooswijk, R. P. Rouhl, A. J. de Louw, W. H. Backes, and J. F. Jansen. Metabolic and functional MR biomarkers of antiepileptic drug effectiveness: A review. *Neurosci Biobehav Rev*, 59:92–9, 2015.

[8] X. Li, R. Ricci, C. H. Large, B. Anderson, Z. Nahas, D. E. Bohning, and M. S. George. Interleaved transcranial magnetic stimulation and fMRI suggests that lamotrigine and valproic acid have different effects on corticolimbic activity. *Psychopharmacology (Berl)*, 209(3):233–44, 2010.

[9] H. Jokeit, M. Okujava, and F. G. Woermann. Carbamazepine reduces memory induced activation of mesial temporal lobe structures: a pharmacological fMRI-study. *BMC Neurol*, 1:6, 2001.

[10] B. Wandschneider, J. Stretton, M. Sidhu, M. Centeno, L. R. Kozák, M. Symms, P. J. Thompson, J. S. Duncan, and M. J. Koepp. Levetiracetam reduces abnormal network activations in temporal lobe epilepsy. *Neurology*, 83(17):1508–1512, 2014.

[11] J. F. Jansen, A. P. Aldenkamp, H. J. Marian Majoie, R. P. Reijs, M. C. de Krom, P. A. Hofman, M. Eline Kooi, K. Nicolay, and W. H. Backes. Functional MRI reveals declined prefrontal cortex activation in patients with epilepsy on topiramate therapy. *Epilepsy Behav*, 9(1):181–5, 2006.

[12] A. De Ciantis, M. Muti, C. Piccolini, M. Principi, A. Di Renzo, R. De Ciantis, D. Frondizi, G. Iannone, P. Ottaviano, and M. Piccirilli. A functional MRI study of language disturbances in subjects with migraine headache during treatment with topiramate. *Neurol Sci*, 29 Suppl 1: S141–3, 2008.

[13] C. L. Yasuda, M. Centeno, C. Vollmar, J. Stretton, M. Symms, F. Cendes, M. A. Mehta, P. Thompson, J. S. Duncan, and M. J. Koepp. The effect of topiramate on cognitive fMRI. *Epilepsy Res*, 105(1-2):250–5, 2013.

[14] M. Rubinov and O. Sporns. Complex network measures of brain connectivity: uses and interpretations. *Neuroimage*, 52(3):1059–69, 2010.

[15] M. P. van den Heuvel, C. J. Stam, R. S. Kahn, and H. E. Hulshoff Pol. Efficiency of functional brain networks and intellectual performance. *J Neurosci*, 29(23):7619–24, 2009.

[16] C. Giessing, C. M. Thiel, A. F. Alexander-Bloch, A. X. Patel, and E. T. Bullmore. Human brain functional network changes associated with enhanced and impaired attentional task performance. *J Neurosci*, 33(14):5903–14, 2013.

[17] M. C. G. Vlooswijk, M. J. Vaessen, J. F. A. Jansen, M. C. F. T. M. de Krom, H. J. M. Majoie, P. A. M. Hofman, A. P. Aldenkamp, and W. H. Backes. Loss of network efficiency associated with cognitive decline in chronic epilepsy. *Neurology*, 77(10):938–944, 2011.

[18] L. Bonilha, A. Tabesh, K. Dabbs, D. A. Hsu, C. E. Stafstrom, B. P. Hermann, and J. J. Lin. Neurodevelopmental alterations of large-scale structural networks in children with new-onset epilepsy. *Hum Brain Mapp*, 35(8):3661–72, 2014.

[19] Z. Haneef, H. S. Levin, and S. Chiang. Brain graph topology changes associated with anti-epileptic drug use. *Brain Connect*, 5(5):284–91, 2015.

[20] T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, R. H. C. Lazeron, N. A. J. Puts, R. A. E. Edden, P. A. M. Hofman, A. J. A. de Louw, W. H. Backes, and J. F. A. Jansen. Glutamate concentrations vary with antiepileptic drug use and mental slowing. *Epilepsy & Behavior*, 64: 200–205, 2016.

[21] S. R. Samarasekera, C. Helmstaedter, and M. Reuber. Cognitive impairment in adults with epilepsy: The relationship between subjective and objective assessments of cognition. *Epilepsy Behav*, 52(Pt A):9–13, 2015.

[22] A. P. Aldenkamp, J. Arends, N. M. de la Parra, and E. J. W. Migchelbrink. The cognitive impact of epileptiform EEG discharges and short epileptic seizures: relationship to characteristics of the cognitive tasks. *Epilepsy & Behavior*, 17(2):205–209, 2010.

[23] D. M. IJff and A. P. Aldenkamp. *Comorbidities of treatment with antiepileptic drugs*, pages 424–36. New York: McGraw-Hill Professional, 2012.

[24] J. Raven, J. C. Raven, and J. H. Court. Manual for Raven's Progressive Matrices and Vocabulary Scales. Section 3: The Standard Progressive Matrices. *San Antonio, TX: Harcourt Assessment*, 2000.

[25] E. Grevers, L. E. Breuer, I. Jff DM, and A. P. Aldenkamp. Mental slowing in relation to epilepsy and antiepileptic medication. *Acta Neurol Scand*, 2015.

[26] E. van Diessen, S. J. Diederen, K. P. Braun, F. E. Jansen, and C. J. Stam. Functional and structural brain networks in epilepsy: what have we learned? *Epilepsia*, 54(11):1855–65, 2013.

[27] K. Murphy, R. M. Birn, and P. A. Bandettini. Resting-state fMRI confounds and cleanup. *Neuroimage*, 80:349–59, 2013.

[28] E. T. Bullmore and D. S. Bassett. Brain graphs: graphical models of the human brain connectome. *Annu Rev Clin Psychol*, 7:113–40, 2011.

[29] M. Rubinov and O. Sporns. Weight-conserving characterization of complex functional brain networks. *Neuroimage*, 56(4):2068–79, 2011.

[30] M. W. Lammers, Y. A. Hekster, A. Keyser, H. Meinardi, W. O. Renier, and H. van Lier. Monotherapy or polytherapy for epilepsy revisited: a quantitative assessment. *Epilepsia*, 36(5):440–446, 1995.

[31] F. Verhage. *Intelligentie en leeftijd: Onderzoek bij Nederlanders van twaalf tot zevenenzeventig jaar*. Van Gorcum Assen, 1964.

[32] W. Alpherts and A. P. Aldenkamp. FePsy: the iron psyche. *Heemstede: Instituut voor Epilepsiebestrijding*, 1994.

[33] C. Giessing and C. M. Thiel. Pro-cognitive drug effects modulate functional brain network organization. *Front Behav Neurosci*, 6:53, 2012.

[34] A. Hahn, G. S. Kranz, R. Sladky, S. Ganger, C. Windischberger, S. Kasper, and R. Lanzenberger. Individual diversity of functional brain network economy. *Brain Connect*, 5(3):156–65, 2015.

[35] E. Santarnecchi, S. Rossi, and A. Rossi. The smarter, the stronger: Intelligence level correlates with brain resilience to systematic insults. *Cortex*, 64:293–309, 2015.

[36] M. Mula. Topiramate and cognitive impairment: evidence and clinical implications. *Ther Adv Drug Saf*, 3(6):279–89, 2012.

[37] E. van Diessen, W. J. Zweiphenning, F. E. Jansen, C. J. Stam, K. P. Braun, and W. M. Otte. Brain network organization in focal epilepsy: A systematic review and meta-analysis. *PLoS One*, 9(12):e114606, 2014.

[38] I. Kida, A. J. Smith, H. Blumenfeld, K. L. Behar, and F. Hyder. Lamotrigine suppresses neurophysiological responses to somatosensory stimulation in the rodent. *Neuroimage*, 29(1):216–24, 2006.

Part II

Methodological studies

Chapter 5

Glutamate quantification by PRESS or MEGA-PRESS: accuracy, repeatability, and concordance

T. M. van Veenendaal, W. H. Backes, F. C. G. van Bussel, R. A. E. Edden, N. A. J. Puts, A. P. Aldenkamp, J. F. A. Jansen, *Submitted*

Abstract

Objective: While PRESS is often employed to measure glutamate concentrations, MEGA-PRESS enables simultaneous Glx (glutamate and glutamine) and GABA measurements. This study aimed to compare accuracy, repeatability, and concordance of different approaches for glutamate quantification at 3T to aid future studies in their selection of the appropriate sequence and quantification method.

Methods: Nine phantoms with different glutamate and glutamine concentrations and five healthy participants were scanned twice to assess respectively the accuracy and repeatability of measurements with PRESS and MEGA-PRESS. To assess concordance between the different methods, results from 95 human participants were compared. PRESS, MEGA-PRESS, and the MEGA-PRESS OFF spectra were analyzed with both LCModel and Gannet.

Results: *In vitro*, excellent agreement was shown between actual and measured glutamate concentrations for all measurements ($r>0.98$). *In vivo* CVs were better for PRESS (2.9%) than MEGA-PRESS (4.9%) and MEGA-PRESS OFF (4.2%). However, the concordance between the sequences was low (PRESS and MEGA-PRESS OFF, $r=0.3$) to modest (MEGA-PRESS versus MEGA-PRESS OFF, $r=0.8$).

Conclusion: Both PRESS and MEGA-PRESS can be employed to measure *in vivo* glutamate concentrations, although PRESS shows a better repeatability. Comparisons between *in vivo* glutamate measures of different sequences however need to be interpreted cautiously.

Introduction

Clinical studies show increasing interest in *in vivo* measurements of glutamate levels, the most abundant excitatory neurotransmitter in the central nervous system [1, 2]. Altered glutamate concentrations have been observed in several neurological and psychiatric brain diseases, such as epilepsy [3] or schizophrenia [4], and are related to cognitive or behavioral functioning [5]. Besides its function as neurotransmitter, glutamate is involved in several metabolic pathways and its concentration has been shown to be related to excitatory activity [1].

In vivo measurements of glutamate levels are enabled by proton MR spectroscopy (^{1}H-MRS). However, due to J-coupling, the resonance frequency of glutamate resolves with a split pattern. Furthermore, its peaks are overlapped by other metabolites such as N-acetyl-aspartate and glutamine, making it challenging to measure glutamate levels, despite its high abundance in the brain [6]. As glutamate is especially difficult to disentangle from its precursor glutamine, the estimates of the two metabolites are often combined 'Glx'. However, the Glx approach makes the results of studies investigating glutamate ambiguous to interpret as possible shifts in balances between glutamate and glutamine may remain undetected [2, 7].

Dedicated sequences have been developed to assess *in vivo* glutamate concentrations, including spectral editing of glutamate with multiple quantum coherence methods [8–10] or TE averaged point-resolved spectroscopy (TE averaged PRESS) [2, 11]. Unfortunately, these sequences have several disadvantages: scan times are often too long for clinical applications, they might not be available or difficult to use on clinical MR scanners, or they only enable glutamate measurements, but no other possibly interesting neurometabolites. Glutamate concentrations are therefore frequently derived with commonly applied localized single voxel ^{1}H-MRS such as PRESS, possibly with optimized TE for measuring glutamate [2]. To distinguish glutamate from other, overlapping metabolites, prior knowledge fitting can be applied, as for instance implemented in LCModel [12].

Recently, glutamate measurements have often been acquired with MEGA-PRESS [13, 14]. MEGA-PRESS is a J-editing technique commonly used to measure the main inhibitory neurotransmitter γ-aminobutyric acid (GABA), which cannot be derived from standard PRESS scans due to overlapping metabolites. MEGA-PRESS includes editing pulses, selectively editing the GABA signal at 1.9 ppm, which refocuses the GABA signal at 3 ppm [15]. Also off-resonance spectra are acquired, including an editing pulse at 7.46 ppm, which is not expected to have an effect on the spectrum. The GABA concentration can be derived by subtracting these "OFF" spectra from the "ON" spectra (i.e. with editing pulse at 1.9 ppm). Although not originally designed for this purpose, MEGA-PRESS has

also been used to estimate glutamate or Glx concentrations [16–19]. Glutamate and glutamine are chemically related to GABA and are known to co-edit with the MEGA-PRESS sequence, resulting in an additional Glx peak in the difference spectrum. Due to the editing, this Glx signal is no longer overlapped by other metabolites (in contrast with the PRESS sequence) and the area under this peak can simply be integrated to have an estimation of the Glx concentration. Some authors claim that this peak constitutes only glutamate [18, 20, 21], while others claim that glutamate and glutamine can be distinguished in the analysis of the spectra by using prior knowledge fitting [22].

Both PRESS and MEGA-PRESS sequences provide opportunities to investigate glutamate and glutamine concentrations. In addition, glutamate can also be acquired using only the spectra from the MEGA-PRESS acquisition without editing pulse. In clinical studies, time constraints often limit the number of sequences that can be used. When MEGA-PRESS is already applied for GABA quantification, one can wonder whether a separate PRESS scan is still needed for adequate glutamate quantification. However, despite the wide use of these techniques in literature, validation is limited to a small *in vitro* study at 4T and a few reproducibility studies [22–26]. To our knowledge no comparisons between different *in vivo* approaches have been made. This study therefore aims to compare glutamate quantification for PRESS and MEGA-PRESS both in phantom as well as in *in vivo* with measurements at 3T, and to investigate the claim that the Glx peak constitutes mainly glutamate, to aid future clinical studies in their selection of appropriate sequences and analysis methods.

Methods

Approach

This study consisted of three different experiments. The first experiment, a phantom study, aimed to determine and compare the accuracy of PRESS and MEGA-PRESS for the quantification of glutamate (and glutamine) concentrations. The claim that the Glx peak signal from the MEGA-PRESS spectrum constitutes predominantly glutamate was also tested [20]. The second experiment aimed to assess the *in vivo* repeatability of the different methods. The third experiment aimed to assess concordance of glutamate (and glutamine) quantification of different methods in a large population.

Table 5.1. Procedures for MR spectroscopy.

Acquired spectrum	Sequence details	Analysis
PRESS	PRESS sequence: TE/TR: 35/2000 ms, 128 averages, VAPOR water suppression Separately acquired scan without water suppression: TE/TR: 35/2000 ms, 128 averages Total scan duration: 5 min	LCModel
MEGA-PRESS	MEGA-PRESS sequence: TE/TR 68/2000 ms, 320 averages, editing pulses (bandwidth 50Hz) at 1.9 (ON) and 7.46 ppm (OFF) interleaved in 40 blocks, MOIST water suppression Separately acquired scan without water suppression: TE/TR 68/2000 ms, 8 averages Total scan duration: 11 min	LCModel, Gannet
MEGA-PRESS OFF	MEGA-PRESS sequence: TE/TR 68/2000 ms, 160 averages, editing pulse at 7.46 ppm, MOIST water suppression Separately acquired scan without water suppression: TE/TR 68/2000 ms, 8 averages Total scan duration: 11 min	LCModel

MR measurements

All MR measurements were performed on a 3T MR scanner (Philips Achieva TX, Philips Healthcare, the Netherlands) with a 32 channel head coil. MR spectroscopy included a MEGA-PRESS and a PRESS (Table 5.1) sequence for both the phantoms and human participants. PRESS was performed with a TE of 35 ms, the shortest TE at our clinical scanner and commonly applied in clinical studies. The PRESS and MEGA-PRESS measurements were performed directly after each other (with and without water suppression) (Table 1). The order of these scans was alternated between the phantoms in Experiment 1, while in Experiment 2 and 3, all measurements started with PRESS. A 3x3x3 cm^3 voxel-of-interest was placed, which was not repositioned between the scans. In the human participants, this voxel was positioned in the occipito-parietal lobe (Figure 5.1), which was located on the T1-weighted image (1 mm cubic voxel, fast field echo sequence, TR/TE/TI 8.1/3.7/1008.6 ms, 170 sagittal slices).

Spectral analysis

The MRS measurements resulted in three different spectra: the PRESS spectrum and the two subspectra from the MEGA-PRESS scan, namely a spectrum with an editing pulse at 1.9 ppm ("ON") and a spectrum with an editing pulse at 7.46 ppm, which is not expected to have an effect on the spectrum ("OFF", i.e. a PRESS spectrum with TE 68 ms). Difference spectra were obtained by subtrac-

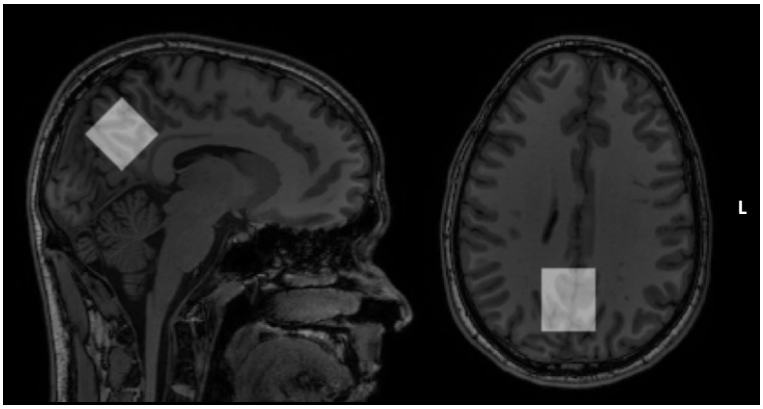

Figure 5.1. Voxel placement in the *in vivo* experiments. L: left

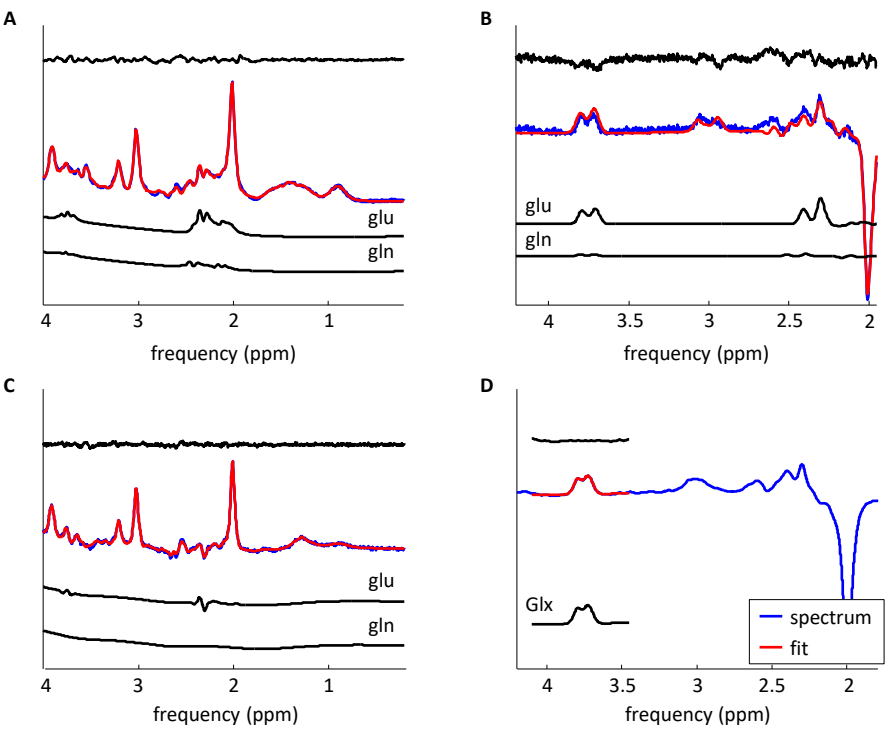

Figure 5.2. Examples of the different spectra measured *in vivo*. LCModel analyses of the PRESS (A), MEGA-PRESS (B), and MEGA-PRESS OFF scans (C), which show the measured spectrum (blue), total fit (red), individual glutamate (glu) and glutamine (gln) estimates (black, on bottom), and residuals (black, on top). An example of the Gannet analysis of a MEGA-PRESS scan is displayed in d, in which the spectrum (after frequency and phase corrections), Glx fit, and residuals are shown.

tion of "ON" and "OFF" spectra. The PRESS spectrum, MEGA-PRESS OFF, and MEGA-PRESS difference spectra were all analyzed with LCModel version 6.3-1L (Figure 5.2), which analyzes the spectrum as a linear combination of different simulated metabolite spectra [12]. Simulated basis sets were applied (kindly provided by Dr. Provencher), with sixteen simulated metabolites in the analyses of the PRESS and MEGA-PRESS OFF spectra, and six in the analyses of the MEGA-PRESS spectra. In addition, thirteen simulated basis spectra were added to account for macromolecule and lipid contributions. For the phantom measurements, only simulated spectra of glutamate, glutamine, GABA, creatine, inositol, N-acetyalaspartate, and phosphorylcholine were included in the basis set. Settings of LCModel were as described in the LCModel manual [27]: spectra were analyzed from 0.2 to 4.0 ppm for PRESS and MEGA-PRESS OFF and from 1.95 to 4.2 ppm for the MEGA-PRESS scans. Eddy current correction was included in all analyses; the MEGA-PRESS option was applied in the analyses of the MEGA-PRESS data. Frequency and phase correction is implemented in LCModel and applied to the averaged spectrum. Gannet (version 2.0) was applied to estimate the surface area of the 'Glx peak' in the MEGA-PRESS difference spectrum using a double Gaussian fit (Figure 5.2) [28]. Preprocessing in Gannet included frequency and phase correction, including spectral registration between each block of subspectra [29, 30]. The concentrations were considered relative to the separately acquired water signal. No correction for tissue composition was included, as only within-subject variations within the same voxel were compared. All statistical analyses described in the following paragraphs were performed with R version 3.2.2 [31].

Experiment 1

Nine 350 mL phantom solutions were made with a stock solution as described in the LCModel manual [27], including sodium azide, DSS, sodium formate, and a phosphate buffer. The stock solution furthermore contained physiological concentrations of creatine (7.8 mM), myo-Inositol (5.9 mM), N-acetylaspartate (10.3 mM) and choline (1.7 mM) [6]. Glutamate, glutamine and GABA were added to the stock solution in five different concentrations, which were based on physiological concentrations, i.e. approximately 6.0-12.5 mM, 3.0-5.8 mM, and 1.3-1.9 mM for glutamate, glutamine, and GABA, respectively [6]. The concentrations varied from 0 (1 phantom), within the physiological range (3 phantoms), to twice the mean value of the physiological range (1 phantom, Table 5.2). The pH of the solutions was set to 7.2 using NaOH.

All phantoms were prepared on the day of the MR experiments. Each phantom was measured twice and temperature-controlled at 36.5-38.0°C using a water bath

Table 5.2. Glutamate, glutamine and γ-aminobutyric acid (GABA) concentrations in the different phantoms.

Phantom	Glutamate (mM)	Glutamine (mM)	GABA (mM)
1	18.2	0.0	0.0
2	0.0	9.2	0.0
3	0.0	0.0	3.5
4	6.2	4.5	1.9
5	6.0	6.1	1.6
6	9.0	2.9	1.9
7	9.0	6.0	1.3
8	12.1	3.0	1.5
9	12.0	4.6	1.3

which was continuously refilled with water heated outside the MR room.

To assess the accuracy, the Pearson correlation coefficient was calculated between actual and measured concentrations of glutamate, glutamine, Glx, and GABA. To assess relative contributions of glutamate, glutamine, and GABA concentrations to the Glx peak in the MEGA-PRESS spectrum, linear regression analysis was applied with the measured Glx peak as dependent variable, and present metabolite concentrations as independent variables. In both analyses, mean concentrations of the two repeated measurements were considered.

Experiment 2

To assess *in vivo* repeatability, five healthy participants (age 29±4 year, four male) were recruited. All participants provided written informed consent before participation in the experiment. The MR spectroscopy protocol was directly repeated to limit physiological variations within the participants. In this experiment, the coefficient of variation (CV) was calculated, which was defined as the $\frac{1}{n}\sum_{i=1}^{n} SD_i/x_i$, with i the participant, n the number of participants (i.e. n=5), SD_i the sample standard deviation of the two repeated measurements, and x_i the average of the two measurements. Additionally, Bland-Altman analyses were performed. Therefore, the standard deviation of the difference between the two repeated measurements (s_c) was computed [32]. The 95% limits of agreement (LoAs) were equal to $1.96 \cdot s_c$.

Three different CVs and LOAs for glutamate, glutamine, Glx, and GABA were obtained based on the PRESS, MEGA-PRESS OFF, and MEGA-PRESS difference spectra, all analyzed with LCModel. In addition, a fourth CV and LoA (only Glx and GABA) was calculated based on the MEGA-PRESS difference spectrum analyzed with Gannet.

Experiment 3

Concordance between the different methods was tested using MRS data of 106 participants, who were included in a study on type 2 diabetes mellitus [33]. The study population comprised 41 healthy participants, 47 patients with type 2 diabetes, and 18 patients with metabolic syndrome (which is considered a prestage of type 2 diabetes). Fifty-nine percent of the participants were male (41% female) and the age of all participants was 63±8 years (mean±std). The study was approved by the local medical ethical committee and all participants gave their written informed consent. This study is registered at clinicaltrials.gov (with identifier NCT01705210).

To test concordance between the three spectra, the Pearson's correlation coefficient was calculated. Similar to Experiment 2, the correlation between four different measurements was calculated. In addition, correlations between glutamate and Glx estimates were calculated, as Glx estimates are often used as a proxy for glutamate estimates.

Results

Experiment 1

All estimated concentrations can be found in the Appendix. With a Pearson's correlation coefficient larger than or equal to 0.98 in all measurements ($p<0.001$), the individual LCModel estimates showed a high correlation with the prepared glutamate, glutamine, Glx, and GABA concentrations, except for the GABA estimates in PRESS (r=0.55, $p=0.12$) and MEGA-PRESS OFF (r=0.88, $p=0.002$).

The regression analysis, performed to assess the relative contribution of glutamate, glutamine, and GABA to the Glx peak measured with MEGA-PRESS, showed significant associations of both glutamate and glutamine with this Glx peak (Table 5.3). The regression coefficients were comparable, indicating that the estimated glutamate:glutamine ratio is similar in the Glx peak as the actual proportion. GABA was not significantly associated to the Glx peak.

Experiment 2

As five participants were scanned twice, 5x2 PRESS and MEGA-PRESS spectra were obtained. All acquired spectra had sufficient quality for further analyses (visually inspected) and had an SNR≥20, although not all metabolites could be detected in all spectra. In the PRESS scan, the CRLBs were ≤5 for glutamate and Glx, and ≤20 for glutamine. Glutamate and Glx had CRLBs ≤7 and ≤5 in MEGA-PRESS scans (OFF and difference spectra, respectively). Quantification of

Table 5.3. Results of the linear regression analysis with the measured Glx peak (Glx as measured with MEGA-PRESS and analyzed with Gannet) as dependent variable, and the known phantom concentrations as dependent variables. The table shows the (not standardized) regression coefficient (β) and its 95%-confidence interval.

	β	95%-CI
[Glutamate]	0.106*	0.093–0.119
[Glutamine]	0.087*	0.061–0.112
[GABA]	-0.001	-0.040–0.037

*$p \leq 0.001$

glutamine failed in seven out of ten difference spectra, and in one out of ten OFF spectra (zero glutamine detected), which were subsequently excluded from further analyses. In none of the participants two glutamine estimations from MEGA-PRESS difference were available, therefore these results were further disregarded. In the OFF spectra of four remaining participants, the CRLB of glutamine was between 20 and 30. GABA could only be estimated from the MEGA-PRESS difference spectra and had a CRLB$\leq$6 in the LCModel analyses. In the MEGA-PRESS scan, the average absolute drift of the water peak between two subsequent blocks was $1.7 \cdot 10^{-3} \pm 0.6 \cdot 10^{-3}$ ppm.

CVs and LoAs of the *in vivo* experiments are displayed in Table 5.4. The lowest CV was shown in the PRESS spectrum, while the CVs were comparable between the MEGA-PRESS difference and OFF spectra. The Bland-Altman analyses showed, in case of glutamate, the most narrow LoA for the PRESS spectrum and larger LoAs for both MEGA-PRESS spectra (Figure 5.3). For Glx, the LoA was most narrow in the MEGA-PRESS spectrum (Table 5.4). Except for the MEGA-PRESS spectra, all LoAs were more narrow for glutamate than for Glx.

Experiment 3

Data of 95 participants was included in this experiment. In five of the 106 participants, no complete PRESS/MEGA-PRESS data sets were acquired. In another six participants, data was excluded for insufficient spectral quality (n=3) and because of high drifts in the MEGA-PRESS scans (n=3, >2 ppm difference in resonance frequency of the water peak between two subsequent blocks).

In the included 95 participants, CRLBs of glutamate and Glx were $\leq$6 in the PRESS scan and $\leq$20 in both MEGA-PRESS scans. Again, quantification of glutamine was difficult, with only 72 participants with a CRLB$\leq$20 in the PRESS scan, nineteen in the MEGA-PRESS scan and five in the MEGA-PRESS OFF

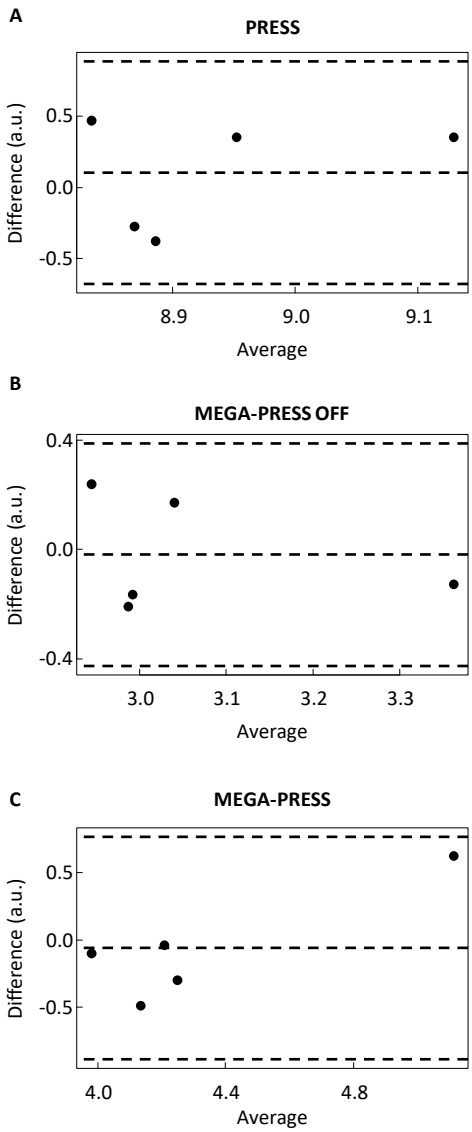

Figure 5.3. Bland-Altman plots showing the *in vivo* repeatability of glutamate estimates with PRESS (A), MEGA-PRESS OFF (B) and MEGA-PRESS (C).

Table 5.4. Repeatability of *in vivo* measurements (n=5) with PRESS, MEGA-PRESS OFF, and MEGA-PRESS. The Limits of Agreement are displayed relative to the mean concentrations (as can be found in the Appendix). All concentrations are estimated with LCModel, unless indicated differently.

	glutamate	glutamine	Glx	GABA+[a]
Coefficient of variation				
PRESS	2.9%	8.8%	3.1%	NR
MEGA-PRESS OFF	4.2%	9.7%[b]	4.5%	NR
MEGA-PRESS	4.9%	NR	4.3%	6.1%
MEGA-PRESS (Gannet)	-	-	5.2%	8.1%
Limits of Agreement				
PRESS	1±9%	-3±34%	0±12%	NR
MEGA-PRESS OFF	-1±13%	-12±24%[b]	-3±13%	NR
MEGA-PRESS	-1±19%	NR	-6±9%	-2±21%
MEGA-PRESS (Gannet)	-	-	-5±21%	-9±31%

NR: no reliable estimates could be given for these metabolites [a]GABA including possible macromolecules, which can add up to 50% of the total GABA+ signal [34]. [b]based on four out of five participants

scan. Therefore, glutamine was disregarded in further analyses. The average absolute drift of the water peak was $4.2 \cdot 10^{-3} \pm 4.9 \cdot 10^{-3}$ ppm between two subsequent blocks in the MEGA-PRESS scan.

The Pearson's correlation coefficients, calculated between glutamate and Glx as measured with the different methods, are displayed in Table 5.5. The concordance between PRESS and MEGA-PRESS was low, albeit more beneficial for glutamate than for Glx (Figure 5.4). Furthermore, the concordance between the two spectra (difference and OFF) acquired with the MEGA-PRESS sequence was high, and better than between the PRESS and MEGA-PRESS sequence. Strikingly is the high correlation of glutamate and Glx in the MEGA-PRESS OFF scan, compared with this correlation in the PRESS or MEGA-PRESS scans. Finally, the correlation of the Glx estimate from GANNET and LCModel is modest (r=0.65, p<0.001), but was expected to be higher considering the fact that exactly the same data were analyzed with different programs.

Discussion

In this study, three different experiments were performed to assess the quantification of glutamate. In all these experiments, four different combinations of acquisition and analysis methods were compared to distinguish glutamate from the other metabolites: (i) a PRESS sequence, of which the spectrum was analyzed with

Table 5.5. Pearson's correlation coefficients between the different *in vivo* applied MRS methods. The concentrations are analyzed with LCModel (except for Glx_{MP} (Gannet), which was analyzed with Gannet) and expressed relative to the water signal. Results are ordered according to the sequence used, indicated by the shaded boxes.

	Glu_{PRESS}	Glx_{PRESS}	Glu_{MP}	Glx_{MP}	Glx_{MP} (Gannet)	Glu_{MPO}	Glx_{MPO}
Glu_{PRESS}	-	0.83**	0.40**	0.39**	0.21	0.35*	0.37*
Glx_{PRESS}	0.83**	-	0.25	n.s.	n.s.	0.27*	0.24
Glu_{MP}	0.40**	0.25	-	0.86**	0.63**	0.78**	0.79**
Glx_{MP}	0.39**	n.s.	0.86**	-	0.65**	0.75**	0.86**
Glx_{MP} (Gannet)	0.21	n.s.	0.63**	0.65**	-	0.87**	0.83**
Glu_{MPO}	0.35*	0.27*	0.78**	0.75**	0.87**	-	0.94**
Glx_{MPO}	0.37*	0.24	0.79**	0.86**	0.83**	0.94**	-

n.s.: non-significant correlation, *: $p<0.01$, **: $p<0.0001$
MP: MEGA-PRESS, MPO: MEGA-PRESS OFF

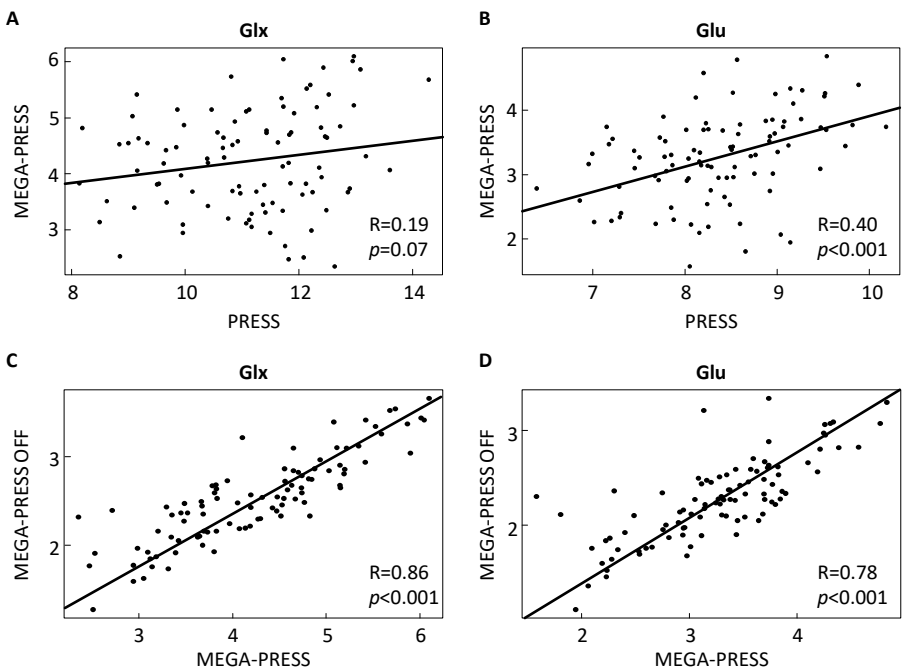

Figure 5.4. Both the Glx (A) as the glutamate (B) concentrations (relative to water) measured *in vivo* with PRESS and MEGA-PRESS show a low correlation, despite being measured in the same participants and directly after each other. Higher correlations were measured between the MEGA-PRESS and MEGA-PRESS OFF estimates of Glx (C) and glutamate (D).

LCModel, (ii) a MEGA-PRESS sequence with analysis in LCModel, (iii) the off-spectrum from MEGA-PRESS, analyzed in LCModel, and (iv) MEGA-PRESS with analysis of the 'Glx' peak using Gannet. The first experiment, a phantom study, showed a high correlation between measured and actual glutamate concentrations. The second experiment showed better CVs and LoAs for *in vivo* glutamate measurements with PRESS than with MEGA-PRESS OFF or MEGA-PRESS. When comparing the quantitative estimates themselves of the different methods *in vivo*, as performed in the third experiment, the correlation between the methods was only modest (varying from r=0.3 for PRESS and MEGA-PRESS OFF to r=0.8 between MEGA-PRESS and MEGA-PRESS OFF).

Previous studies already showed sufficient accuracy in glutamate measurements with 30 ms PRESS and the MEGA-PRESS OFF spectra at 4T [23]. Also appropriate repeatability [24] and reproducibility values of PRESS [25, 26] or MEGA-PRESS [22] for measuring glutamate in humans was shown previously. Corresponding to our results, the reproducibility of glutamate and Glx was comparable [22, 24], while a much better reproducibility of glutamate than of glutamine was shown (only with PRESS [25]). This latter might be explained by the higher *in vivo* glutamate than glutamine concentrations, leading to better signal-to-noise ratios in glutamate measurements.

The lack of strong concordance between the PRESS and MEGA-PRESS estimates found in our study is surprising. A possible explanation is the attribution of other molecules to the glutamate and glutamine signal, most likely macromolecules. In general, contribution of macromolecules is lower at longer TEs due to the relatively short T2 relaxation times of macromolecules [35]. This could also explain the low concordance between PRESS (TE=35) and both MEGA-PRESS (TE=68) and MEGA-PRESS OFF (TE=68), while the concordance between the latter two was much higher. Less contamination by macromolecules can therefore be expected in the MEGA-PRESS than in PRESS spectra, although it is unknown how the editing pulse affects the relative ratio between Glx and macromolecules. In MEGA-PRESS, up to 50% of the GABA+ peak constitutes macromolecules [34], but it has been suggested that no macromolecules are present in the Glx peak [22]. More research is needed to assess the contributions of these macromolecules to the Glx signals in both PRESS as MEGA-PRESS.

A secondary question in this study was, whether the Glx peak measured with a MEGA-PRESS sequence constitutes mainly glutamate, as claimed by others [18, 21, 36]. Our phantom results show that both glutamate and glutamine contribute strongly to this Glx peak. Previous *in vivo* work, concluded that the Glx peak in MEGA-PRESS constitutes primarily glutamate, based on a comparison between the Glx measure from MEGA-PRESS and glutamate and combined glutamate and

glutamine measures from CT-PRESS [20], and argued that Glx measurements can be interpreted as glutamate. However, as the glutamate and Glx concentration are inherently correlated, the independent contribution of glutamate to Glx cannot be estimated *in vivo*. Thus, solely measuring the 'Glx' peak does not yield an unambiguous estimate of glutamate, and Glx alterations in patient studies should not automatically be attributed to changes in glutamate only.

An important issue is that some authors report reservations whether short-echo PRESS scans can reliably separate the glutamate and glutamine signals. For instance, Henry et al. [23] showed in simulations (at 4T) that with increasing linewidths, rises in glutamate estimations are accompanied with large drops in glutamine estimations, suggesting unreliable separation. Contrasting, glutamate concentrations estimated at 4T were similar to those at 7T, with higher reliability at 7T [37]. Our phantom experiment also showed good separation of the two signals, but these results cannot be directly translated to *in vivo* experiments as linewidths might be smaller in phantoms than *in vivo*. The high correlation of glutamate and Glx, as seen in MEGA-PRESS OFF (r=0.94), likely indicates that glutamine was (potentially incorrectly) attributed to glutamate in these measurements.

Although in all three experiments, all scans were made in a single session without repositioning of the participant (or phantom), it is possible that small differences in voxel position due to head movement might have occurred. This could explain some random error, but is not expected to cause the lack of concordance seen in experiment 3, as the MEGA-PRESS scan was performed directly after PRESS scan.

The main limitation of this study is the gap between phantom experiments, in which actual concentrations are known, and the *in vivo* situation which includes unknown confounding factors such as possible macromolecules and movement artifacts. This is illustrated by the low concordance shown *in vivo* between the different methods despite their good accuracy and repeatability. Furthermore, we chose to only compare PRESS with TE=35 ms, with MEGA-PRESS with TE=68 ms, as these settings are commonly applied to measure glutamate/Glx. However, also other TEs can be used, which might be more beneficial to measure glutamate [2]. Another limitation is that glutamate and glutamine concentrations were only assessed within normal range. It is possible that one method is more favorable when glutamate concentrations are below this range, as might be the case in some conditions. Finally, only LCModel and Gannet were considered in this study, while other analysis software (Tarquin, JMRUI [38, 39]) are available as well.

Conclusion

In conclusion, both PRESS as MEGA-PRESS, in combination with prior knowledge fitting such as implemented in LCModel, enable reliable glutamate measurements. This means that researchers interested in both GABA and glutamate do not require additional PRESS measurements for glutamate, albeit at the cost of a lower repeatability. In case MEGA-PRESS is applied, it is recommended to use the difference spectrum for glutamate detection, as the OFF spectrum does not seem to separate glutamate and glutamine correctly. Further studies are prompted into the effects of macromolecules on glutamate quantification.

Acknowledgements

This work was supported by The Netherlands Organization for Scientific Research (NWO) and The Netherlands Organization for Health Research and Development (ZonMw) [grant number 916.11.059].

References

[1] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[2] S. Ramadan, A. Lin, and P. Stanwell. Glutamate and glutamine: a review of in vivo MRS in the human brain. *NMR Biomed*, 26(12):1630–46, 2013.

[3] O. Petroff. *Biochemistry for Magnetic Resonance Spectroscopy*, chapter 13, pages 333–384. Academic Press, 2005.

[4] E. M. Poels, L. S. Kegeles, J. T. Kantrowitz, D. C. Javitt, J. A. Lieberman, A. Abi-Dargham, and R. R. Girgis. Glutamatergic abnormalities in schizophrenia: a review of proton MRS findings. *Schizophr Res*, 152(2-3):325–32, 2014.

[5] G. Ende. Proton magnetic resonance spectroscopy: Relevance of glutamate and GABA to neuropsychology. *Neuropsychol Rev*, 25(3):315–25, 2015.

[6] V. Govindaraju, K. Young, and A. A. Maudsley. Proton NMR chemical shifts and coupling constants for brain metabolites. *NMR in Biomedicine*, 13(3):129–153, 2000.

[7] Jr. Novotny, E. J., R. K. Fulbright, P. L. Pearl, K. M. Gibson, and D. L. Rothman. Magnetic resonance spectroscopy of neurotransmitters in human brain. *Ann Neurol*, 54 Suppl 6:S25–31, 2003.

[8] H. K. Lee, A. Yaman, and O. Nalcioglu. Homonuclear J-refocused spectral editing technique for quantification of glutamine and glutamate by 1H NMR spectroscopy. *Magnetic resonance in medicine*, 34(2):253–259, 1995.

[9] J. W. Pan, G. F. Mason, G. M. Pohost, and H. P. Hetherington. Spectroscopic imaging of human brain glutamate by water-suppressed J-refocused coherence transfer at 4.1 T. *Magnetic resonance in medicine*, 36(1):7–12, 1996.

[10] R. B. Thompson and P. S. Allen. A new multiple quantum filter design procedure for use on strongly coupled spin systems found in vivo: its application to glutamate. *Magnetic resonance in medicine*, 39(5):762–771, 1998.

[11] R. Hurd, N. Sailasuta, R. Srinivasan, D. B. Vigneron, D. Pelletier, and S. J. Nelson. Measurement of brain glutamate using TE-averaged PRESS at 3T. *Magn Reson Med*, 51(3):435–40, 2004.

[12] S. W. Provencher. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. *Magnetic Resonance in Medicine*, 30(6):672–679, 1993.

[13] Y. Takei, K. Fujihara, M. Tagawa, N. Hironaga, J. Near, M. Kasagi, Y. Takahashi, T. Motegi, Y. Suzuki, Y. Aoyama, N. Sakurai, M. Yamaguchi, S. Tobimatsu, K. Ujita, Y. Tsushima, K. Narita, and M. Fukuda. The inhibition/excitation ratio related to task-induced oscillatory modulations during a working memory task: A multtimodal-imaging study using MEG and MRS. *Neuroimage*, 128:302–15, 2016.

[14] K. Cohen Kadosh, B. Krause, A. J. King, J. Near, and R. Cohen Kadosh. Linking GABA and glutamate levels to cognitive skill acquisition during development. *Hum Brain Mapp*, 36(11): 4334–45, 2015.

[15] N. A. Puts and R. A. Edden. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog Nucl Magn Reson Spectrosc*, 60:29–41, 2012.

[16] S. Kakeda, Y. Korogi, J. Moriya, N. Ohnari, T. Sato, S. Ueno, N. Yanagihara, M. Harada, and T. Matsuda. Influence of work shift on glutamic acid and gamma-aminobutyric acid (GABA): evaluation with proton magnetic resonance spectroscopy at 3T. *Psychiatry Research: Neuroimaging*, 192(1):55–59, 2011.

[17] U. Dydak, Y.-M. Jiang, L.-L. Long, H. Zhu, J. Chen, W.-M. Li, R. A. E. Edden, S. Hu, X. Fu, and Z. Long. In vivo measurement of brain GABA concentrations by magnetic resonance spectroscopy in smelters occupationally exposed to manganese. *Environmental health perspectives*, 119(2):219, 2011.

[18] M. Cleve, A. Gussew, and J. R. Reichenbach. In vivo detection of acute pain-induced changes of GABA+ and Glx in the human brain by using functional 1H MEGA-PRESS MR spectroscopy. *Neuroimage*, 105:67–75, 2015.

[19] F. Riese, A. Gietl, N. Zölch, A. Henning, R. O'Gorman, A. M. Kälin, S. E. Leh, A. Buck, G. Warnock, and R. A. E. Edden. Posterior cingulate γ-aminobutyric acid and glutamate/glutamine are reduced in amnestic mild cognitive impairment and are unrelated to amyloid deposition and apolipoprotein E genotype. *Neurobiology of aging*, 36(1):53–59, 2015.

[20] D. C. Shungu, X. Mao, M. Gu, M. S. Milak, N. Weiduschat, D. Mayer, D. Spielman, J. J. Mann, and L. S. Kegeles. 'Glx' measured by J-editing/MEGA-PRESS is primarily 'pure' glutamate... or is it? In *Proc Int Soc Magn Reson Med*, volume 21, page 3985, 2013.

[21] C. de la Fuente-Sandoval, F. Reyes-Madrigal, X. Mao, P. Leon-Ortiz, O. Rodriguez-Mayoral, R. Solis-Vivanco, R. Favila, A. Graff-Guerrero, and D. C. Shungu. Cortico-striatal GABAergic and glutamatergic dysregulations in subjects at ultra-high risk for psychosis investigated with proton magnetic resonance spectroscopy. *Int J Neuropsychopharmacol*, 19(3), 2015.

[22] R. L. O'Gorman, L. Michels, R. A. Edden, J. B. Murdoch, and E. Martin. In vivo detection of GABA and glutamate with MEGA-PRESS: reproducibility and gender effects. *J Magn Reson Imaging*, 33(5):1262–7, 2011.

[23] M. E. Henry, T. L. Lauriat, M. Shanahan, P. F. Renshaw, and J. E. Jensen. Accuracy and stability of measuring GABA, glutamate, and glutamine by proton magnetic resonance spectroscopy: a phantom study at 4 Tesla. *J Magn Reson*, 208(2):210–8, 2011.

[24] I. Hancu. Optimized glutamate detection at 3T. *J Magn Reson Imaging*, 30(5):1155–62, 2009.

[25] P. G. Mullins, H. Chen, J. Xu, A. Caprihan, and C. Gasparovic. Comparative reliability of proton spectroscopy techniques designed to improve detection of J-coupled metabolites. *Magn Reson Med*, 60(4):964–9, 2008.

[26] F. Schubert, J. Gallinat, F. Seifert, and H. Rinneberg. Glutamate concentrations in human brain using single voxel proton magnetic resonance spectroscopy at 3 Tesla. *Neuroimage*, 21(4):1762–71, 2004.

[27] S. W. Provencher. LCModel & LCMgui user's manual. *LCModel Version 6.3-1H*, 2014.

[28] R. A. Edden, N. A. Puts, A. D. Harris, P. B. Barker, and C. J. Evans. Gannet: A batch-processing tool for the quantitative analysis of gamma-aminobutyric acid-edited MR spectroscopy spectra. *J Magn Reson Imaging*, 40(6):1445–52, 2014.

[29] C. J. Evans, N. A. Puts, S. E. Robson, F. Boy, D. J. McGonigle, P. Sumner, K. D. Singh, and R. A. Edden. Subtraction artifacts and frequency (mis-)alignment in J-difference GABA editing.

J Magn Reson Imaging, 38(4):970–5, 2013.

[30] J. Near, R. Edden, C. J. Evans, R. Paquin, A. Harris, and P. Jezzard. Frequency and phase drift correction of magnetic resonance spectroscopy data by spectral registration in the time domain. *Magn Reson Med*, 73(1):44–50, 2015.

[31] R Core Team. R: A language and environment for statistical computing, 2015.

[32] J. M. Bland and D. G. Altman. Statistical methods for assessing agreement between two methods of clinical measurement. *The lancet*, 327(8476):307–310, 1986.

[33] F. C. van Bussel, W. H. Backes, P. A. Hofman, N. A. Puts, R. A. E. Edden, M. P. van Boxtel, M. T. Schram, C. D. Stehouwer, J. E. Wildberger, and J. F. A. Jansen. Increased GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning. *Medicine (Baltimore)*, 95 (36):e4803, 2016.

[34] A. D. Harris, N. A. Puts, P. B. Barker, and R. A. Edden. Spectral-editing measurements of GABA in the human brain with and without macromolecule suppression. *Magn Reson Med*, 74(6):1523–9, 2015.

[35] C. Cudalbu, V. Mlynarik, and R. Gruetter. Handling macromolecule signals in the quantification of the neurochemical profile. *J Alzheimers Dis*, 31 Suppl 3:S101–15, 2012.

[36] M. S. Milak, C. J. Proper, S. T. Mulhern, A. L. Parter, L. S. Kegeles, R. T. Ogden, X. Mao, C. I. Rodriguez, M. A. Oquendo, R. F. Suckow, T. B. Cooper, J. G. Keilp, D. C. Shungu, and J. J. Mann. A pilot in vivo proton magnetic resonance spectroscopy study of amino acid neurotransmitter response to ketamine treatment of major depressive disorder. *Mol Psychiatry*, 21(3):320–7, 2016.

[37] I. Tkac, G. Oz, G. Adriany, K. Ugurbil, and R. Gruetter. In vivo 1H NMR spectroscopy of the human brain at high magnetic fields: metabolite quantification at 4T vs. 7T. *Magn Reson Med*, 62(4):868–79, 2009.

[38] M. Wilson, G. Reynolds, R. A. Kauppinen, T. N. Arvanitis, and A. C. Peet. A constrained least-squares approach to the automated quantitation of in vivo 1H magnetic resonance spectroscopy data. *Magnetic Resonance in Medicine*, 65(1):1–12, 2011.

[39] D. Stefan, F. Di Cesare, A. Andrasescu, E. Popa, A. Lazariev, E. Vescovo, O. Strbak, S. Williams, Z. Starcuk, M. Cabanas, D. van Ormondt, and D. Graveron-Demilly. Quantitation of magnetic resonance spectroscopy signals: the jMRUI software package. *Measurement Science and Technology*, 20(10):104035, 2009.

Appendix

Measured concentrations of three experiments relative to the water concentration (mean±sd, in arbitrary units).

	Glutamate	Glutamine	Glx	GABA+[a]
Experiment 1[b]				
PRESS	26.5±0.4	12.0±0.6	26.5±0.4	1.6±0.1
MEGA-PRESS OFF	9.2±0.7	3.5±0.3	9.2±0.7	1.1±0.2
MEGA-PRESS	11.7±0.2	5.1±0.4	11.7±0.2	2.1±0.1
MEGA-PRESS (Gannet)	-	-	2.1±0.04	1.4±0.01
Experiment 2				
PRESS	8.9±0.2	2.3±0.5	11.2±0.5	NR
MEGA-PRESS OFF	3.1±0.2	0.5±0.1	3.5±0.1	NR
MEGA-PRESS	4.3±0.4	NR	4.5±0.6	1.7±0.4
MEGA-PRESS (Gannet)	-	-	1.6±0.2	1.8±0.3
Experiment 3				
PRESS	8.4±0.8	2.8±0.8	11.1±1.3	NR
MEGA-PRESS OFF	2.3±0.4	NR	2.5±0.5	NR
MEGA-PRESS	3.3±0.7	NR	4.2±0.9	1.9±0.4
MEGA-PRESS (Gannet)	-	-	1.3±0.2	1.3±0.3

[a]GABA + additional macromolecules, which can add up to 50% of the total GABA signal. No macromolecules were present in Experiment 1, the phantom experiment.

[b]Only averages of the 'maximum phantoms' are displayed; i.e. phantom 1 for glutamate and Glx, phantom 2 for glutamine, and phantom 3 for GABA.

NR: no reliable estimates could be given for these metabolites

Chapter 6

High field imaging of large-scale neurotransmitter networks: proof of concept and initial application to epilepsy

T. M. van Veenendaal, W. H. Backes, D. H. Y. Tse, T. W. J. Scheenen, D. W. Klomp, P. A. M. Hofman, R. P. W. Rouhl, M. C. G. Vlooswijk, A. P. Aldenkamp, J. F. A. Jansen, *Submitted*

Abstract

Objective: Many clinical neuroimaging studies focus on altered structural or functional brain connectivity. However, these studies cannot provide direct information on the defective neurons or the related neurotransmitter imbalance, which underlies abnormal neuronal activity. To assess additional information about underlying large-scale metabolic deficits affecting neuronal functioning, we introduce the concept of 'neurotransmitter networks' and evaluate this in patients with epilepsy.

Methods: Glutamate, GABA and NAA networks were computed across a group of fifteen healthy participants and a group of ten patients with localization-related epilepsy. Each participant underwent 7T MR scanning, comprising T1-weighted imaging and MR spectroscopic imaging. The neurometabolite concentrations were measured in 30 brain regions. Neurometabolite networks were constructed by assessing coordinated spatial variations in neurometabolite concentrations across individuals. Two areas were considered connected when the neurometabolite concentrations for these areas were significantly correlated across the participants.

Results: For the healthy participants, high quality data were available in 21 of 30 brain areas and 21%, 19%, and 26% of the possible connections showed a significant correlation for glutamate, GABA, and NAA, respectively. When comparing networks from patients with epilepsy to those from healthy participants, patients had significantly more stronger connections for GABA and glutamate.

Conclusion: In this first study of neurotransmitter networks, it was demonstrated that interregional correlations of glutamate, GABA, and NAA measurements can be conceptualized as networks. The increased glutamate and GABA connectivity in patients with epilepsy might indicate disrupted neurotransmitter balance, and should be further explored to increase insights into the pathophysiology of this disease.

Introduction

The brain can be considered a network, existing of multiple interconnected brain areas with various functions [1]. Several studies have shown that measures of connectivity between these areas can be associated with cognitive functions [2, 3], and that the connections are affected in several neurological diseases [4, 5]. Characterization of brain networks gained large interest in both neuroscience and neurological studies and different methods are currently being employed. First of all, methods such as functional MRI and EEG can be applied to assess the so-called 'functional networks': areas are linked together and characterized based on simultaneous brain activity. Other methods characterize brain networks based on structural connectivity, by employing diffusion MRI, thereby visualizing fiber bundles in the brain [6], or over individuals assessing shared distributions of cortical thickness [7].

One particular disease that is often studied using network theory is localization-related epilepsy (i.e. epilepsy with a presumed focal structural cause that cannot be identified historically or be seen with current imaging techniques). Although traditionally considered a focal disease, studies applying functional MRI and diffusion MRI have provided convincing evidence that localization-related epilepsy exhibits profound alterations in both local and distributed functional and structural networks [8]. Proper neuronal functioning and signal trafficking across distributed brain regions also rely on the release and presence of (electro)chemical components, in particular neurotransmitters. Functional and diffusion MRI cannot provide direct information on defective neurons or the associated neurotransmitter disbalance, which underlies abnormal neuronal activity, an essential feature of seizures. Insights into the neurotransmitter network dysfunction in localization-related epilepsy might be of great value to eventually better understand the neuronal network characteristics of epilepsy and also other brain diseases.

Proton MR Spectroscopy (^{1}H-MRS) enables the non-invasive detection and quantification of metabolite concentrations in the brain, thereby offering a window on brain cell metabolism [9]. Traditionally, neurometabolites such as N-acetylaspartate (NAA; marker of neuronal loss) and creatine (Cr; marker of energy metabolism) are being measured. Aberrant levels of these neurometabolites have been found in epilepsy, but also other neurological diseases or in aging [10, 11]. More advanced studies also focus on measurements of the inhibitory and excitatory neurotransmitters GABA and glutamate/glutamine, which can be associated with neural activity [12]. However, these studies traditionally consider only local metabolite concentrations, while healthy brain functioning does not only rely on individual brain areas, but also requires proper signal trafficking, and thus relations

between distant brain areas [13]. Using high-field ^{1}H-MRS imaging at 7.0T, it is possible to obtain a snapshot of the spatial distribution of GABA and glutamate with a high (mL) spatial resolution.

Therefore, the concept of 'neurotransmitter networks' is introduced in this study. This new method relates to the assessment of coordinated spatial variations in neurotransmitter concentrations in the brain across individuals, and might be able to provide additional information on the underlying metabolic changes which affect neuronal functions. We primarily focussed on glutamate and GABA, due to their roles as important excitatory and inhibitory neurotransmitters in the brain. Additionally, NAA networks were considered, as NAA is the neurometabolite that is easiest to measure, although not directly involved in signalling. We assessed the construction and first applicability of 'neurotransmitter networks'. The concept is first applied healthy participants, and subsequently compared between patients with epilepsy and healthy participants.

Methods

Study procedures

Two groups of participants, healthy volunteers and patients with epilepsy, were included. The exclusion criteria for both groups were all contraindications for MR scanning (such as metal implants or pregnancy), and a medical history with neurological diseases. Additional exclusion criteria for the patients with epilepsy were MRI visible lesions (seen on clinical 3T scans), changes in antiepileptic drugs (medication or dose) in the last twelve months, or a seizure frequency higher than once a month.

All participants provided written informed consent before participation. Ethical approval for this study was obtained from the medical ethical committee academic hospital Maastricht/Maastricht University, and the study was registered at the Dutch Trial Register with registration number NTR4878.

Each participant underwent a 7 Tesla MR examination (Magnetom, Siemens Healthineers, Erlangen, Germany) with a 32-channel head coil. The scanning procedure included whole brain T1-weighted imaging (MP2RAGE [14], TR/TE 4500/2.39 ms, TI1/TI2 900/2750 ms, FOV 173x230x230 mm^3, cubic voxel size 0.9x0.9x0.9 mm^3), a whole brain fluid attenuation inversion recovery (FLAIR) sequence (TR/TE 8000/303 ms, TI 2330ms, FOV 166.4x224x256 mm^3, cubic voxel size 0.8x0.8x0.8 mm^3), and an MRSI acquisition. For the latter, a semi-LASER sequence was applied, which combines conventional RF pulses for slice excitation with orthogonal adiabatic refocusing pulses for volume selection [15]. Frequency

offset corrected inversion (cFOCI) pulses were included in this sequence to limit chemical shift artifacts [16]. Other parameters were TR/TE 5520/38 ms, VAPOR water suppression, FOV 150x150x100 mm^3 (Figure 6.1), and voxel size 9.4x9.4x12.5 mm^3 (1.1 mL).

Five of the healthy controls were scanned twice, with a seven-day interval, to assess the inter-scan reproducibility of the MRSI measures. Both T1-weighted and FLAIR images were checked by a neuroradiologist (P.A.M.H.) for abnormalities.

Data analysis

Metabolite concentrations

Before constructing the metabolite networks, the metabolite concentrations per brain area were computed. For this purpose, information from the anatomical scan and the spectra were preprocessed and analyzed (Figure 6.2). With the MP2RAGE sequence, images were obtained for two inversion times: TI1 (GRETI1) an TI2 (GRETI2), which were combined to create a quantitative longitudinal relaxation time T1-weighted image [14]. The GRETI2 scan was skull stripped using the brain extraction tool (BET) of FMRIB Software Library (FSL, version 5.0.1) [17, 18]. The T1 map was segmented in gray matter, white matter, and cerebrospinal fluid with FAST, part of FSL [19].

To parcellate the brain into a number of standard areas, the atlas was transformed to the skull stripped T1-weighted image (Figure 6.2). The atlas was defined by thirty non-overlapping brain areas (Table 6.1) and was created in MNI space, by combining information from the Harvard-Oxford cortical and subcortical atlases and the ICBM 2009c nonlinear symmetric template [20–22]. The skull stripped T1 maps were non-linearly transformed to the MNI brain using FNIRT (FSL, version 5.0.1) [23–25]. The inverse non-linear transformation was then applied to transform an atlas to each individual brain.

Metabolite spectra were analyzed using LCModel (version 6.3) with a simulated basis set of 21 metabolites (Vespa [26], Figure 6.1). Default simulated basis spectra from LCModel were applied to account for lipids and macromolecules. Voxelwise spectra were excluded when the signal-to-noise ratio (SNR) was below 20, the Cramér-Rao lower bounds (CRLBs) of the N-acetylaspartate plus N-acetylaspartylglutamate concentrations (tNAA) was higher than 3, the CRLB of creatine plus phosphocreatine (tCr) was higher than 10, or the CRLB of GABA or glutamate was higher than 20. The spectra were also excluded if the total gray and white matter fraction of the corresponding voxel was lower than 50%. All concentrations were expressed relative to the tCr concentration of the corresponding voxel.

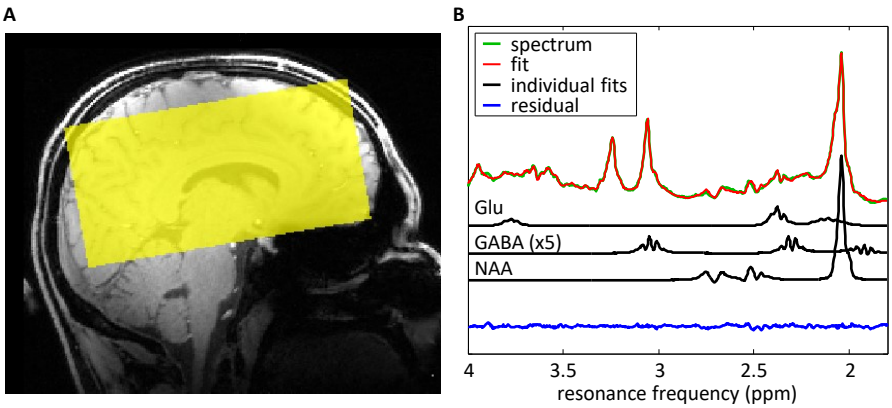

Figure 6.1. A. Field-of-view of the MRSI image. B. Typical example of a measured spectrum with the quantitative (LCModel) fit, and individual glutamate (glu), GABA and N-acetylaspartate (NAA) fits.

Each MRSI voxel first aligned with the T1-weighted image, and then assigned to the predefined atlas area that showed largest overlap with the voxel. The average concentration per area was corrected for gray and white matter content, age and sex with linear regression analysis, in which the metabolite concentrations were used as the dependent variable (across all participants), and the gray/white matter content, age and sex as independent variables. Gray/white matter content was defined as the fraction of gray matter divided by the gray plus white matter fraction. The corrected metabolite concentrations were equal to the residuals of the linear regression analysis (Equation 6.1):

$$\text{Conc} = \beta_0 + \beta_1 \cdot \frac{\text{GM}}{\text{GM} + \text{WM}} + \beta_2 \cdot \text{age} + \beta_3 \cdot \text{sex} + \text{Conc}_{\text{corr}} \tag{6.1}$$

with Conc the measured (i.e. uncorrected) metabolite concentration per area, Conc_{corr} the corrected metabolite concentration, GM the percentage gray matter and WM the percentage white matter. The Conc_{corr} can be interpreted as the metabolite concentration corrected for variations in gray/white matter content, age, and sex over the participants. The regression coefficients are displayed as β.

As final preprocessing step, standardized concentrations were calculated over all healthy participants (Equation 6.2):

$$\text{Conc}_{\text{scaled}} = \text{Conc}_{\text{corr}} - \frac{\text{mean}(\text{Conc}_{\text{corr}})}{\text{sd}(\text{Conc}_{\text{corr}})} \tag{6.2}$$

with $\text{Conc}_{\text{corr}}$ the corrected and $\text{Conc}_{\text{scaled}}$ the standardized and corrected metabolite concentrations, and sd the standard deviation.

Table 6.1. Atlas areas, the number of included spectroscopic voxels per area, and percentage of subjects with corresponding data (based on the combined patient and control group).

Area	No. of voxels (mean±standard deviation)		Percentage subjects with good-quality data	
Hemisphere	Left	Right	Left	Right
Thalamus	4.3±2.1	4.5±1.8	100%	100%
Basal Ganglia	4.3±2.7	3.8±2.3	92%[a]	92%
Hippocampus/ Amygdala	1.2±1.2	0.7±0.8	64%[a]	44%[a]
Prefrontal GM	13.9±7.3	11.9±6.3	100%	100%
Prefrontal WM	19.8±6.7	20.3±8.0	100%	100%
Insula	7.5±3.8	7.6±4.5	100%	88%[a]
Premotor GM	6.4±4.8	4.9±4.4	96%	84%[a]
Premotor WM	11.4±4.7	12.6±4.6	100%	100%
Temporal GM	3.1±3.6	2.1±2.9	68%[a]	56%[a]
Temporal WM	11.0±4.9	10.5±6.0	100%	96%[b]
Parietal GM	12.0±4.7	13.4±5.2	100%	100%
Parietal WM	16.8±4.6	15.4±3.8	100%	100%
Occipital GM	9.4±6.1	10.5±7.7	96%[b]	92%[b]
Occipital WM	0.9±1.2	1.0±1.1	48%[a]	64%[a]
Cingulate gyrus	5.4±3.1	9.2±2.9	100%	100%

[a]These areas were excluded in the final analyses, because not all participants had good-quality data in these areas.

[b]These areas were additionally excluded in the comparison between patients and healthy participants, because of missing patient data.

Brain metabolite networks and measures

A connection between two brain areas was considered present, when the neurometabolite concentrations of these two areas correlated significantly over the subjects. We assume that areas support concerted brain activity if an increased neurometabolite concentration in one area is accompanied with an altered concentration of that neurometabolite in another area. Areas with correlated neurometabolite concentrations among participants are therefore considered 'metabolically connected'. To obtain the neurometabolite connectivity matrix, the Pearson's correlation coefficient between the standardized concentrations of each pair of areas was calculated. The weighted connectivity between pairs of areas was equal to the correlation coefficient ρ if the correlation was below a certain statistically significant threshold (i.e., $p<0.05$) and 0 otherwise.

A prerequisite for the analyses is that none of the participants had areas without good-quality data. Therefore, some areas and participants were excluded from the final analysis (Table 6.1).

Group comparison

To compare the neurometabolite networks in the patients with epilepsy with the control-group, two basic network characteristics were evaluated: the density (i.e. the fraction of actual connections divided by total number of possible connections), and the average network strength. The density was calculated in a range of varying thresholds (varying from ρ=0.1–0.9) for both subject groups. In this comparison, the Pearson correlation coefficient ρ was applied as threshold, as it is independent of group size, in contrast with the p-value of a correlation.

For the average network strength, a fixed set of nodes was defined, which was based on the combined results of the two groups per neurometabolite. The density of the network was set at 0.2, which corresponded to a threshold of approximately p=0.05 in the healthy subject-group (Figure 6.3A). Subsequently, metabolite networks were created for patients and controls, using the fixed network, and the connection strengths of these networks were statistically compared using a binomial test. For this, the number of connections that were stronger in the patients with epilepsy, and the number of connections stronger in the healthy participants were tallied. The probability of this distribution is approximately binomially distributed, thus the probability of the found distribution can be computed by:

$$Pr(k) = \binom{n}{k} p^k (1-p)^{n-k} \tag{6.3}$$

with k the number of connections that is larger in one group, n the total number of connections, and p the probability that a single connection is higher in one group than the other (p=0.5). The p-value is then given by the cumulative distribution:

$$Pr(x \leq k) = \sum_{i=0}^{k} \binom{n}{i} p^i (1-p)^{n-i} \tag{6.4}$$

In these analyses, the correction for gray/white matter, age and sex, and the standardization (as described in Equation 6.2), was applied over the pooled group containing both controls and patients.

To test the robustness for within-subject variations, this analysis was repeated with replacing the data from the 5 healthy participants who were scanned twice with the 'second measurements'.

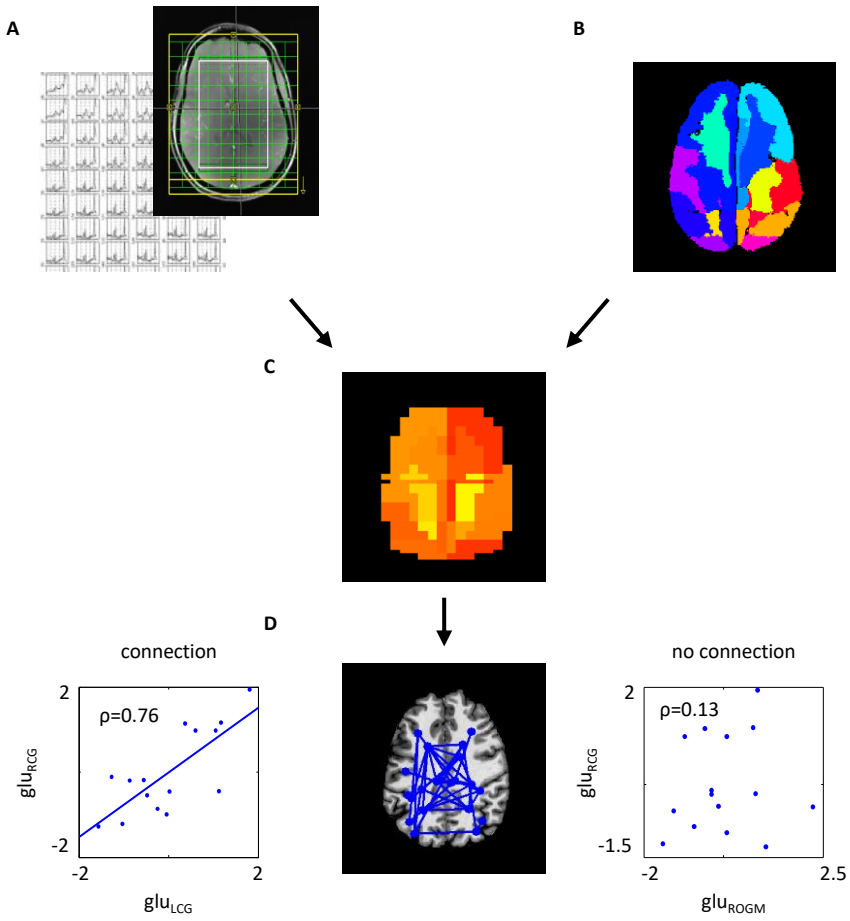

Figure 6.2. Analysis of the metabolite networks. The spectra were first analyzed in LCModel (A) and aligned with the structural image (B), and mean metabolite concentrations were calculated per area (C). Connections between areas were defined as correlated neurometabolite concentrations (D). Glu: standardized glutamate concentration; RCG: right cingulate gyrus; LCG: left cingulate gyrus; ROGM: right occipital gray matter

Results

Study population

Thirty participants were included in this study (20 participants without and 10 with epilepsy). Data of two healthy participants were excluded because of an overall low spectral quality. Therefore, data of 18 healthy participants (10/8 male/female, age 39.6±16.8 years, age range: 22–65) were considered for further analyses. All ten patients with localization-related epilepsy (7/3 male/female, age 40.1±16.5 years, age range: 20–69 years) were included in these analyses. In none of the participants, epileptogenic abnormalities were identified in the 7T images by our expert neuroradiologist.

Metabolite concentrations

Concentrations of GABA, glutamate, and NAA were measured in thirty different brain areas (Table 6.1). No significant differences were observed for the GABA, glutamate, or NAA concentrations between the patients and controls in any of these areas (Student's t-test, $p>0.05$). The glutamate and NAA concentrations were negatively associated with age in respectively 77% and 73% of the brain areas (linear regression analysis, $p<0.05$), while the GABA concentrations were only negatively associated with age in 20% of the brain areas (linear regression analysis, $p<0.05$).

As no missing data were allowed in the network analyses, data from fifteen healthy participants and 21 areas were included in these analyses (Table 6.1). In case of the analyses in patients, ten patients and eighteen areas were included. The average SNR per area across all participants was 47±8 (mean±SD), while the average CRLBs of glutamate, GABA and NAA were 4.1±0.9, 13.5±0.8, and 2.1±0.2, respectively. The coefficients of variation of the concentration estimates were 8.4±4.2% for glutamate, 11.4±3.8% for GABA, and 7.8±3.1% for NAA (see Appendix).

Organization of metabolic brain networks

Of the possible connections, 21%, 19%, and 26% showed a significant glutamate, GABA, and NAA correlation, respectively, ($p<0.05$) across all fifteen healthy participants (Figure 6.3). The density of the networks is higher than expected purely based on chance (i.e. the p-value) for all three metabolites (Figure 6.4A). The maximum node degree (number of connections per node) was nine; this was observed in the left prefrontal white matter (glutamate) or left parietal white matter (GABA,

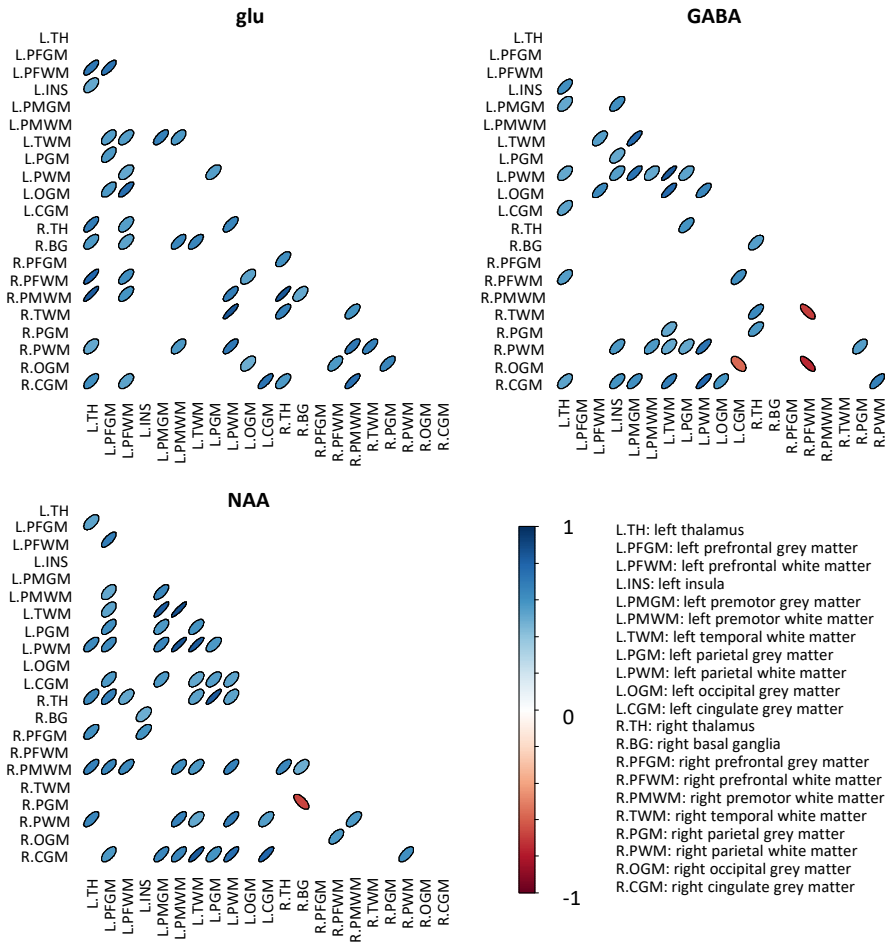

Figure 6.3. Correlation matrices of the glutamate (glu), GABA and N-acetylaspartate (NAA) networks in healthy participants. The shape and color of the ovals indicate the correlations coefficient (ρ) between each pair of areas, i.e. the strength of the connections between those areas and the sign of the correlations (red: negative, blue: positive). Only connections with a $p<0.05$ are displayed. Figures were created in R version 3.2.2 [27, 28].

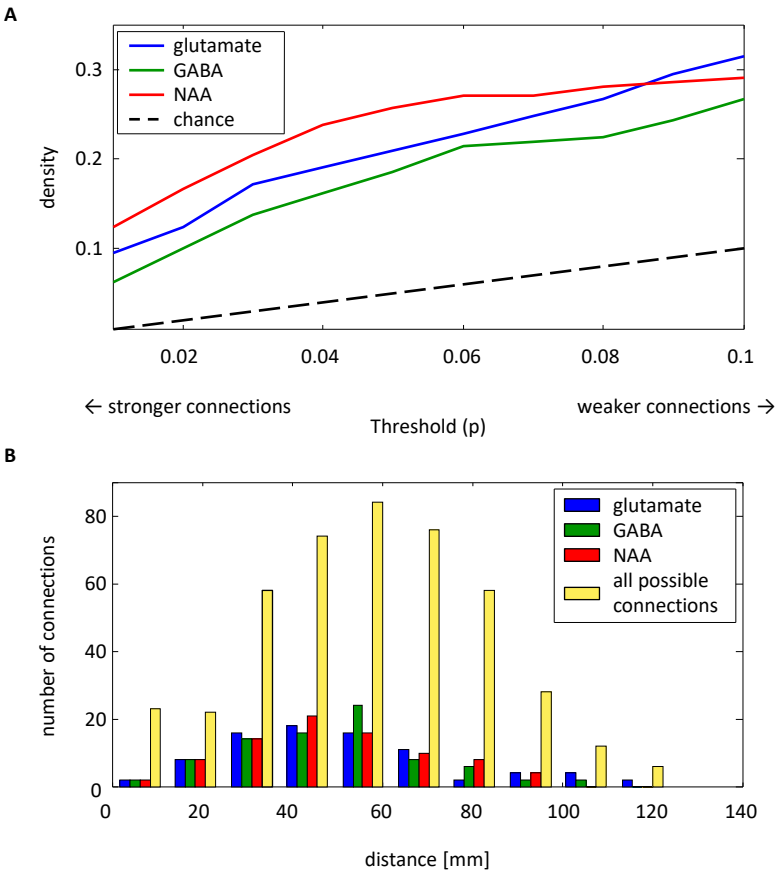

Figure 6.4. Characteristics of the neurometabolite networks. In A, the density of the networks is displayed at different thresholds (i.e. the *p*-value of the correlation). It can be observed that the density of the networks is much higher than would be expected by chance only. B displays the distribution of distances between the nodes, including the distances between all possible networks. The neurometabolite networks include both long- and short distance connections. The density of networks was set to 0.2 for all three metabolites.

Table 6.2. Comparison of network characteristics between patients and controls. The density is given at a threshold of ρ=0.6, whereas the average connection strength is given at a density of 0.2. Because of missing data in the patient group, fewer areas (n=18) were included in these analyses than those described in Figure 6.4.

	Density		Average connection strength	
	Controls[a]	Patients	Controls[a]	Patients
Glutamate	0.15 (0.20)	0.31	0.62 (0.66)	0.61
GABA	0.10 (0.07)	0.22	0.54 (0.47)	0.64
NAA	0.20 (0.19)	0.25	0.65 (0.68)	0.64

[a]Numbers between brackets display the results of the analyses with the five second measurements and give an indication of the robustness for within-subject variations.

NAA). All nodes were connected in the glutamate network, while the GABA network had three unconnected nodes (left and right prefrontal gray matter, and the right premotor white matter). The NAA network had two unconnected nodes (left occipital gray matter and right temporal white matter), and another component (with only two nodes: right prefrontal white matter and right occipital gray matter) which was unconnected to the other nodes. The vast majority of connections was positive: no significant, negative connections were present in the glutamate network, while 11% and 4% of the respectively GABA and NAA connections was negative.

The relation between the number of connections and distance between the areas is illustrated in Figure 6.4. It can be observed that the distances between all areas are roughly normally distributed. The neurometabolite networks include both long- and short distance connections.

Metabolite networks in epilepsy

The density of the glutamate and GABA networks were higher in the patients with epilepsy compared with the healthy controls (Figure 6.5, Table 6.2). Also the connection strength of the GABA networks was higher in the patients than the controls. The average connection strength of the glutamate networks was comparable between the patients and controls. The NAA networks showed a comparable density and node strength in patients and controls. When comparing the individual connections strengths, the patients with epilepsy displayed a larger Pearson correlation coefficient ρ (i.e. strength) compared with the healthy participants in 97 of the 153 glutamate connections (p<0.001), 103 of the 152 GABA connections (p<0.001), and 83 of the 153 NAA connections (p=0.17).

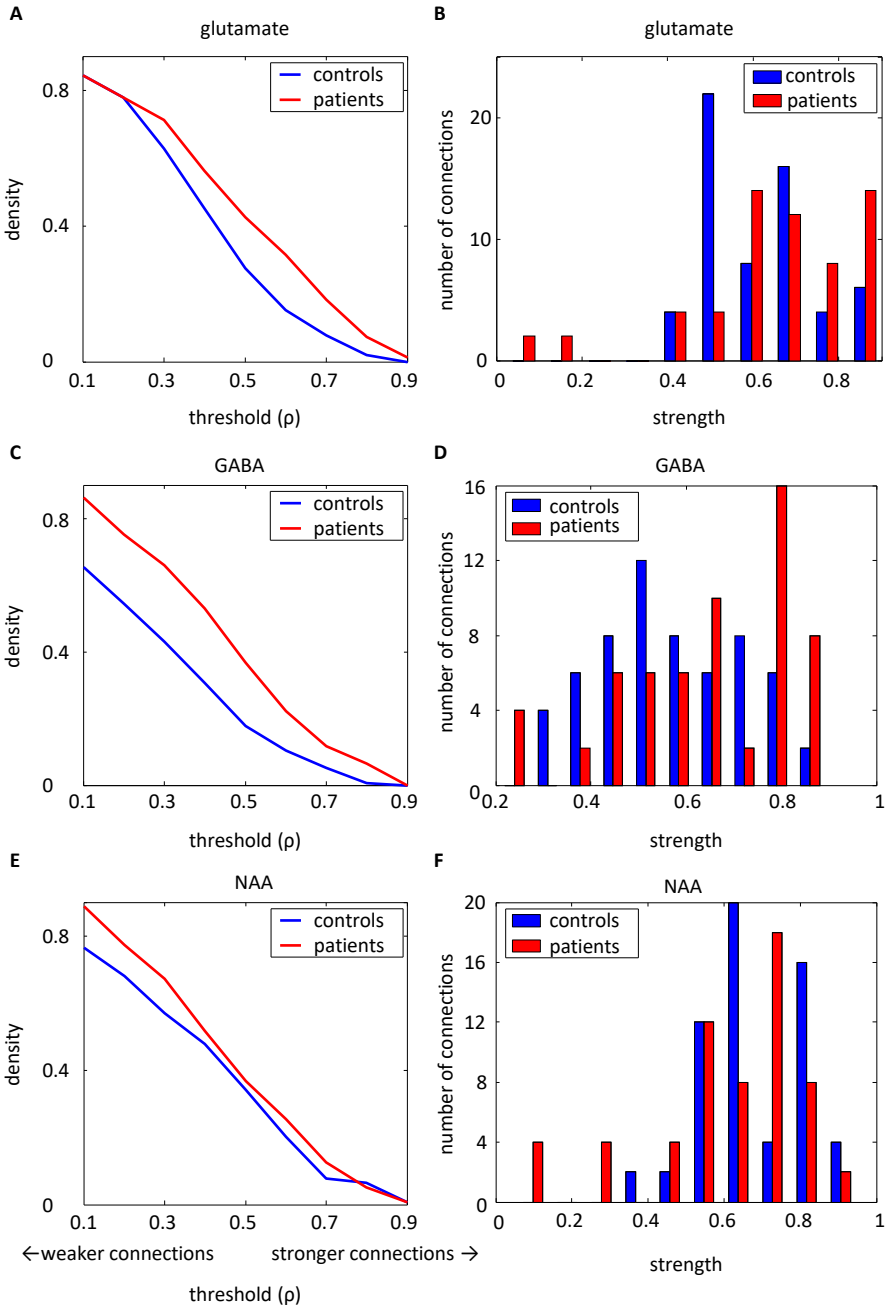

Figure 6.5. Network density in the healthy-subject group and patient group (A, C, E). In both glutamate and GABA networks, the density in patients is higher than that of the healthy participants. B, D, and F show how the distributions of connection strengths of both groups in the glutamate, GABA, and NAA networks. While the glutamate and NAA networks show similar strength distributions, the GABA network shows increased connection strengths in patients with epilepsy.

Discussion

In this study, the concept of neurotransmitter networks was introduced. A spatial relation between glutamate, GABA, and NAA concentrations of different areas was shown, suggesting the presence of neurometabolite networks. These networks included both short-range and long-range connections. Furthermore, some areas were characterized as so-called 'hub areas': areas with many connections to other areas, while other areas showed fewer to no connections. Finally, these networks displayed significantly different properties in patients with epilepsy in comparison with healthy participants.

Interpretation of neurotransmitter networks

An important question is how to interpret these metabolic networks, or, on a smaller scale, metabolic connections. The conceptual idea is that areas that are connected, tend to have similar characteristics, an idea known in social sciences as the 'homophily principle' [29]. This principle has previously been applied to neurosciences, for instance in correlated cortical thickness [7], gene expression in the brain [30] or serotonin-receptor binding measured with positron emission tomography (PET) [31].

Several previous studies already assessed interregional correlations in glutamate, GABA, and NAA concentrations [32–35]. It is difficult to compare their results with ours, as they used different methods (e.g. field strength, brain areas). Our study showed the existence of interregional correlations for all three neurometabolites, but the specific coherence depended on the areas being compared; not all areas showed a correlation, which might explain the contrasting results seen in different studies.

An explanation for the biological mechanisms behind these connections remains speculative. Beside its function as neurotransmitter, glutamate is available as metabolic pools in the neuron and linked to glucose metabolism, and glutamate levels are linked to excitatory activity [9]. GABA, on the other hand, is most likely related to the GABAergic tone (i.e. the level of continuous GABAergic activity, inducing tonic inhibition, which is not necessarily inhibitory activity) [9]. Several studies suggested that neuronal connectivity is related to GABA- and glutamatergic function. At neuronal level, it has been shown that the probability of a connection between two types of neurons, correlates with the average synaptic strength of those two neuron types [36]. At brain level, positive correlations have been shown between glutamate concentrations and functional connectivity, and negative between GABA and functional connectivity [12].

NAA is considered a marker for neuronal integrity and associated with cognitive function [9]. It can be hypothesized that when areas are connected, damage in one brain areas is accompanied with a decreased neuronal integrity of the other brain area.

Although individual connections are important, these connections are not isolated, as there is a constant interaction between different brain areas and their connections. Therefore, it might be beneficial to assess these brain networks as a whole, which was investigated in this study. Previous structural and functional studies found that brain networks appear so-called 'small-world networks', meaning that they combine a high integration as well as segregation in the network [37]. At this moment, such network characteristics could not be assessed, because of the relatively low number of nodes and the presence of both positive and negative weights, but this should be explored in further studies.

Neurotransmitter networks in epilepsy

A higher GABA and glutamate connectivity was shown in patients with epilepsy compared with a healthy control group, as indicated by the number of connections and the strengths of the connections. This increased neurotransmitter connectivity seems to be associated with the pathophysiology in epilepsy, as epileptic seizures likely disrupt neurotransmitter networks. Previous studies showed an increased functional and structural connectivity in patients around the epileptic zone, but a decreased connectivity in other brain areas, and it has been suggested that brain networks are reorganized as a result of recurrent epileptic seizures [38]. Also, antiepileptic drugs might alter glutamate and GABA concentrations [39], and may result in aberrant metabolic brain networks, but these effects were beyond the scope of the current study.

Previous studies showed significant correlations between NAA concentrations in the hippocampus, thalamus, and putamen in patients with temporal lobe epilepsy, while these correlations were absent in healthy subjects [40, 41]. No changes in NAA connectivity were observed in the current study, but this connectivity might be location-specific. While these previous studies studied connectivity between specific brain areas, we choose to study overall brain networks.

Study considerations

This explorative study aimed to introduce the concept of neurotransmitter networks and should be considered as proof-of-concept. Employing a correlation over the group as an outcome measure leads to some methodological considerations, as it restricts the possibilities for statistical testing. This was solved by compar-

ing individual connection strengths between groups. Future, larger studies might adopt statistical methods applied in cortical thickness studies, that employ similar outcome measures, such as permutation tests [42, 43] or methods to calculate individual measures [44]. Unfortunately, the group size in this pilot study is not sufficient to employ these methods. Finally, the applied MRSI sequence has some disadvantages, such as a limited spatial resolution and coverage, and vulnerability to artifacts. Some of these disadvantages could be solved with other sequences, such as GluCEST, which enables whole-brain glutamate measurements with a high resolution [45, 46]. Future studies with larger group sizes and dedicated spectroscopic imaging protocols are thus necessary to evaluate the presented concept in more detail.

Future Implications

The results are expected to provide a better understanding of the role of neurotransmitter dysfunction in epilepsy and other neurodegenerative diseases. Non-invasive imaging of neurotransmitter networks might provide an early biomarker of patients at risk (i.e. before the onset of overt symptoms). Lastly, it could aid the development of novel effective treatment options, and be used to assess the efficacy of pharmacological interventions.

Conclusion

This study presents the novel concept of metabolic brain networks using MR spectroscopic imaging. We showed interregional correlations of glutamate, GABA, and NAA measurements, which can be conceptualized as networks. We also showed the applicability of this concept in patients with epilepsy; however, it might also provide new insights for other neurological diseases.

Acknowledgements

The authors thank Esther Steijvers, Lotty Huijboom, and Chris Wiggins for help with data acquisition, and Vincent Boer for help with metabolite spectra simulations. We also would like to acknowledge Alfons Kessels and Dave Langers for statistical support. This study was funded by the University Fund Limburg/SWOL (S.2014.14).

References

[1] C. J. Stam and E. C. van Straaten. The organization of physiological brain networks. *Clin Neurophysiol*, 123(6):1067–87, 2012.

[2] M. Song, Y. Zhou, J. Li, Y. Liu, L. Tian, C. Yu, and T. Jiang. Brain spontaneous functional connectivity and intelligence. *NeuroImage*, 41(3):1168–76, 2008.

[3] M. P. van den Heuvel, C. J. Stam, R. S. Kahn, and H. E. Hulshoff Pol. Efficiency of functional brain networks and intellectual performance. *J Neurosci*, 29(23):7619–24, 2009.

[4] S. S. Spencer. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*, 43(3):219–227, 2002.

[5] M. D. Fox and M. Greicius. Clinical applications of resting state functional connectivity. *Front Syst Neurosci*, 4:19, 2010.

[6] D. K. Jones. Studying connections in the living human brain with diffusion MRI. *Cortex*, 44(8): 936–52, 2008.

[7] Y. He, Z. J. Chen, and A. C. Evans. Small-world anatomical networks in the human brain revealed by cortical thickness from MRI. *Cereb Cortex*, 17(10):2407–19, 2007.

[8] B. C. Bernhardt, S. Hong, A. Bernasconi, and N. Bernasconi. Imaging structural and functional brain networks in temporal lobe epilepsy. *Front Hum Neurosci*, 7:624, 2013.

[9] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[10] O. Petroff. *Biochemistry for Magnetic Resonance Spectroscopy*, chapter 13, pages 333–384. Academic Press, 2005.

[11] G. Öz, J. R. Alger, P. B. Barker, R. Bartha, A. Bizzi, C. Boesch, P. J. Bolan, K. M. Brindle, C. Cudalbu, and A. Dinçer. Clinical proton MR spectroscopy in central nervous system disorders. *Radiology*, 270(3):658–679, 2014.

[12] N. W. Duncan, C. Wiebking, and G. Northoff. Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans – a review of multimodal imaging studies. *Neurosci Biobehav Rev*, 47C:36–52, 2014.

[13] H. J. Park and K. Friston. Structural and functional brain networks: from connections to cognition. *Science*, 342(6158):1238411, 2013.

[14] J. P. Marques, T. Kober, G. Krueger, W. van der Zwaag, P. F. Van de Moortele, and R. Gruetter. MP2RAGE, a self bias-field corrected sequence for improved segmentation and T1-mapping at high field. *Neuroimage*, 49(2):1271–81, 2010.

[15] T. W. Scheenen, A. Heerschap, and D. W. Klomp. Towards 1H-MRSI of the human brain at 7T with slice-selective adiabatic refocusing pulses. *MAGMA*, 21(1-2):95–101, 2008.

[16] R. J. Ordidge, M. Wylezinska, J. W. Hugg, E. Butterworth, and F. Franconi. Frequency offset corrected inversion (FOCI) pulses for use in localized spectroscopy. *Magnetic resonance in medicine*, 36(4):562–566, 1996.

[17] S. M. Smith. Fast robust automated brain extraction. *Hum Brain Mapp*, 17(3):143–55, 2002.

[18] M. Jenkinson, C. F. Beckmann, T. E. Behrens, M. W. Woolrich, and S. M. Smith. FSL. *NeuroImage*, 62(2):782–90, 2012.

[19] Y. Zhang, M. Brady, and S. Smith. Segmentation of brain MR images through a hidden Markov random field model and the expectation-maximization algorithm. *Medical Imaging, IEEE Transactions on*, 20(1):45–57, 2001.

[20] J. A. Frazier, S. Chiu, J. L. Breeze, N. Makris, N. Lange, D. N. Kennedy, M. R. Herbert, E. K. Bent, V. K. Koneru, and M. E. Dieterich. Structural brain magnetic resonance imaging of limbic and thalamic volumes in pediatric bipolar disorder. *American Journal of Psychiatry*, 162(7): 1256–1265, 2005.

[21] R. S. Desikan, F. Ségonne, B. Fischl, B. T. Quinn, B. C. Dickerson, D. Blacker, R. L. Buckner, A. M. Dale, R. P. Maguire, and B. T. Hyman. An automated labeling system for subdividing the human cerebral cortex on MRI scans into gyral based regions of interest. *Neuroimage*, 31(3):

968–980, 2006.

[22] V. S. Fonov, A. C. Evans, R. C. McKinstry, C. R. Almli, and D. L. Collins. Unbiased nonlinear average age-appropriate brain templates from birth to adulthood. *NeuroImage*, 47:S102, 2009.

[23] M. Jenkinson and S. Smith. A global optimisation method for robust affine registration of brain images. *Medical image analysis*, 5(2):143–156, 2001.

[24] M. Jenkinson, P. Bannister, M. Brady, and S. Smith. Improved optimization for the robust and accurate linear registration and motion correction of brain images. *NeuroImage*, 17(2):825–841, 2002.

[25] J. L. R. Andersson, M. Jenkinson, and S. Smith. Non-linear registration, aka spatial normalisation FMRIB technical report TR07JA2. *FMRIB Analysis Group of the University of Oxford*, 2, 2007.

[26] B. J. Soher, P. Semanchuk, D. Todd, J. Steinberg, and K. Young. VeSPA: integrated applications for RF pulse design, spectral simulation and MRS data analysis. In *Proceedings of 19th Annual Meeting ISMRM*, page 1410, 2011.

[27] R Core Team. R: A language and environment for statistical computing, 2015.

[28] Kevin Wright. *corrgram: Plot a Correlogram*, 2016. R package version 1.10.

[29] Ö. Şimşek and D. Jensen. Navigating networks by using homophily and degree. *Proc Natl Acad Sci U S A*, 105(35):12758–62, 2008.

[30] J. Richiardi, A. Altmann, A.-C. Milazzo, C. Chang, M. M. Chakravarty, T. Banaschewski, G. J. Barker, A. L. W. Bokde, U. Bromberg, and C. Büchel. Correlated gene expression supports synchronous activity in brain networks. *Science*, 348(6240):1241–1244, 2015.

[31] D. Erritzoe, K. Holst, V. G. Frokjaer, C. L. Licht, J. Kalbitzer, F. A. Nielsen, C. Svarer, J. Madsen, and G. Knudsen. A nonlinear relationship between cerebral serotonin transporter and 5-HT(2A) receptor binding: an in vivo molecular imaging study in humans. *J Neurosci*, 30(9):3391–7, 2010.

[32] I. D. Grachev and A. V. Apkarian. Chemical network of the living human brain: Evidence of reorganization with aging. *Cognitive brain research*, 11(2):185–197, 2001.

[33] K. W. Waddell, P. Zanjanipour, S. Pradhan, L. Xu, E. B. Welch, J. M. Joers, P. R. Martin, M. J. Avison, and J. C. Gore. Anterior cingulate and cerebellar gaba and glu correlations measured by (1)H J-difference spectroscopy. *Magn Reson Imaging*, 29(1):19–24, 2011.

[34] N. V. Kraguljac, M. A. Reid, D. M. White, J. den Hollander, and A. C. Lahti. Regional decoupling of N-acetyl-aspartate and glutamate in schizophrenia. *Neuropsychopharmacology*, 37(12):2635–42, 2012.

[35] I. Greenhouse, S. Noah, R. J. Maddock, and R. B. Ivry. Individual differences in GABA content are reliable but are not uniform across the human cortex. *NeuroImage*, 139:1–7, 2016.

[36] X. Jiang, S. Shen, C. R. Cadwell, P. Berens, F. Sinz, A. S. Ecker, S. Patel, and A. S. Tolias. Principles of connectivity among morphologically defined cell types in adult neocortex. *Science*, 350(6264):aac9462, 2015.

[37] E. Bullmore and O. Sporns. Complex brain networks: graph theoretical analysis of structural and functional systems. *Nat Rev Neurosci*, 10(3):186–98, 2009.

[38] D. J. Englot, P. E. Konrad, and V. L. Morgan. Regional and global connectivity disturbances in focal epilepsy, related neurocognitive sequelae, and potential mechanistic underpinnings. *Epilepsia*, 57(10):1546–1557, 2016.

[39] T. M. van Veenendaal, D. M. IJff, A. P. Aldenkamp, P. A. Hofman, M. C. Vlooswijk, R. P. Rouhl, A. J. de Louw, W. H. Backes, and J. F. Jansen. Metabolic and functional MR biomarkers of antiepileptic drug effectiveness: A review. *Neurosci Biobehav Rev*, 59:92–9, 2015.

[40] H. P. Hetherington, R. I. Kuzniecky, K. Vives, O. Devinsky, S. Pacia, D. Luciano, B. Vasquez, S. Haut, D. D. Spencer, and J. W. Pan. A subcortical network of dysfunction in TLE measured by magnetic resonance spectroscopy. *Neurology*, 69(24):2256–2265, 2007.

[41] J. W. Pan, D. D. Spencer, R. Kuzniecky, R. B. Duckrow, H. Hetherington, and S. S. Spencer. Metabolic networks in epilepsy by MR spectroscopic imaging. *Acta Neurol Scand*, 126(6):411–20, 2012.

[42] D. S. Bassett, E. Bullmore, B. A. Verchinski, V. S. Mattay, D. R. Weinberger, and A. Meyer-

Lindenberg. Hierarchical organization of human cortical networks in health and schizophrenia. *J Neurosci*, 28(37):9239–48, 2008.

[43] Y. He, Z. Chen, and A. Evans. Structural insights into aberrant topological patterns of large-scale cortical networks in alzheimer's disease. *J Neurosci*, 28(18):4756–66, 2008.

[44] M. Saggar, S. M. Hosseini, J. L. Bruno, E. M. Quintin, M. M. Raman, S. R. Kesler, and A. L. Reiss. Estimating individual contribution from group-based structural correlation networks. *Neuroimage*, 120:274–84, 2015.

[45] K. Cai, R. P. Nanga, L. Lamprou, C. Schinstine, M. Elliott, H. Hariharan, R. Reddy, and C. N. Epperson. The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7T (1)H-MRS study. *Neuropsychopharmacology*, 37(13):2764–71, 2012.

[46] K. Cai, A. Singh, D. R. Roalf, R. P. Nanga, M. Haris, H. Hariharan, R. Gur, and R. Reddy. Mapping glutamate in subcortical brain structures using high-resolution GluCEST MRI. *NMR Biomed*, 26(10):1278–84, 2013.

Appendix

Coefficient of variation in metabolite concentration per brain area.

Region	glutamate	GABA	NAA
Left hemisphere			
Thalamus	11%	7.4%	11%
Prefrontal GM	7.0%	8.4%	9.8%
Prefrontal WM	6.9%	4.6%	3.3%
Insula	9.4%	11%	9.5%
Premotor GM	20%	20%	13%
Premotor WM	15%	8.6%	12%
Temporal WM	6.7%	7.5%	6.6%
Parietal GM	20%	16%	12%
Parietal WM	16%	3.8%	5.2%
Occipital GM	10%	16%	11%
Cingulate gyrus	11%	11%	6.2%
Right hemisphere			
Thalamus	13%	4.5%	5.5%
Basal Ganglia	8.9%	8.1%	10%
Prefrontal GM	12%	6.2%	7.9%
Prefrontal WM	8.1%	6.6%	6.8%
Premotor WM	10%	6.6%	6.2%
Temporal WM	11%	5.7%	6.0%
Parietal GM	9.7%	6.0%	3.5%
Parietal WM	16%	4.3%	4.4%
Occipital GM	8.5%	10%	9.8%
Cingulate gyrus	11%	4.3%	2.8%

Chapter 7

General discussion

Continuous evaluation of clinical studies, improvement of existing techniques, and explorations of novel measurement techniques are necessary to increase insights into epilepsy and improve its treatment. In this context, a number of clinical and methodological studies were set out in the current thesis, for which the aim was twofold. The first aim was to identify neuronal substrates of cognitive side effects of antiepileptic drugs (AEDs) measured with currently available magnetic resonance (MR) techniques. The second aim was to develop and apply magnetic resonance techniques that may give new insights in the role of neurotransmitters in epilepsy and impaired cognition, an often occurring co-morbidity of epilepsy.

Considering the first aim, a literature review (Chapter 2) and two clinical MR studies on patients with epilepsy were performed, assessing the associations between AED use, cognition, and neurotransmitter concentrations (Chapter 3) or functional brain network organization (Chapter 4). Considering the second aim, two studies have been performed. Chapter 5 describes the results of a study performed on the accuracy, repeatability, and concordance of two MR spectroscopy (MRS) sequences commonly applied to measure concentrations of the neurotransmitter glutamate. In Chapter 6, the spatial cerebral distribution of the neurotransmitters glutamate and GABA, was investigated and the concept of neurotransmitter networks was introduced.

Three questions relevant for these five chapters, were:

(i) How do AEDs affect the brain, and how is that related to cognitive side effects?

(ii) How do local measurements provide information on distal effects and brain networks?

(iii) And how valid are the advanced measurement techniques applied?

The results of the five chapters together will be discussed according to these three questions in the following sections, ending with a general conclusion.

Cognitive side effects of antiepileptic drugs

Current findings

To identify neuronal substrates of cognitive side effects of AEDs, two different techniques were employed: (i) MRS, that enables *in vivo* measurements of glutamate and γ-aminobutyric acid (GABA), which are the most common excitatory and inhibitory neurotransmitters in the brain, respectively, and (ii) functional MR imaging (fMRI), which provides measures indicative of brain activity.

Previous MRS studies showed that AEDs with a known GABAergic mechanism of action, such as topiramate and vigabatrin, increased cerebral GABA concentrations. Effects on glutamate concentrations were assessed by only a few studies so far and are therefore currently not known (Chapter 2). By combining neuropsychological testing with MRS, we showed that lower glutamate concentrations could be linked to cognitive slowing in patients with epilepsy taking AEDs (Chapter 3).

At neuronal scale, AEDs are claimed to suppress (hyper) excitability. However, at brain level fMRI studies showed that brain activity can either be decreased, remain unaffected or can even be increased (Chapter 2). The impact of these effects were AED type dependent and also differed between various brain regions. However, previous studies suggested that not the brain activity per se, but coordinated brain activity between different brain regions, i.e. functional brain networks, are important for cognitive functioning [1–3]. Therefore we performed a study of these functional brain networks and AED use in relation to the existing cognitive problems (Chapter 4). In this study, surprisingly no associations between cognitive slowing and brain network organization were found, and only the small subgroup of patients taking AEDs from the highest risk group (n=4) showed altered network measures compared with patients taking AEDs from the low- or intermediate-risk group. Based on this study, we concluded that the effect of AEDs on functional brain network organization may be subtle and only detectable in patients with more severe cognitive side effects.

Mechanisms of action

Currently, more than twenty different AEDs are available with different mechanisms of action, which can be distinguished in three main mechanisms: GABAergic, glutamatergic, and voltage-gated channels [4]. Cognitive side effects of AEDs are likely a direct effect of these mechanisms [5]. Side effects are often dose-dependent and occur only during the use of AEDs and disappear if AED use is discontinued. It has been suggested that mainly GABAergic mechanisms induce cognitive side effects, but also these effects can be caused by AEDs with other mechanisms of action [6]. For instance, the AED phenytoin targets voltage-gated sodium channels, but is also known to affect attention, memory and mental speed [7]. These factors suggest that cognitive side effects are a result of attenuated neuronal activity with AED use. The observed association between lower glutamate concentrations and cognitive slowing supports this hypothesis, as glutamate concentrations are likely related to excitatory activity [8]. However, specific mechanisms might attribute to specific cognitive complaints: GABAergic mechanisms are hypothesized to affect attention, while glutamatergic mechanisms might have a negative effect on learning

and memory due to their interference with NMDA receptor, important in learning and memory [6].

Methodological considerations

The distinction between cognitive problems due to epilepsy itself or due to treatment is a major problem when assessing cognitive side effects of AEDs. Epilepsy is a very heterogeneous disease, with a large variety in causes (varying from tumors to genetic defects), affected regions in the brain, number and type of seizures, and age of onset [9]. All these effects might affect cognition differently [10], and also the selection of appropriate AEDs is based on some of these factors [9]. A disadvantage of the observational nature of the studies described in this thesis is therefore the possible confounding effects of the various characteristics of the epilepsy itself.

Another consideration about the design of the study is the distinction of the AEDs in low, intermediate, and high-risk categories, irrespective of the mechanism of action. This can be justified by the cognitive side effects, but the effects of AEDs on neurotransmitter concentrations, or on functional brain organization, might depend on the mechanism of action. More studies are therefore necessary to assess these associations in individual AEDs.

Clinical implications and future directions

AED treatment aims to achieve seizure freedom without inducing adverse effects. Unfortunately, this treatment is often accompanied with side effects, of which cognitive problems are an important category [11]. Currently, we are neither able to predict nor to explain which patient will suffer from side effects. More insights into this topic might improve AED treatment in two ways: first, it might help clinical decision-making if a neurologists knows beforehand if a patient is vulnerable to develop cognitive side effects, and second, it might provide guidance in the search for new AEDs with less cognitive side effects.

The associations of brain glutamate levels, AED use, and cognition described in Chapter 3 might give rise to more focused studies to predict cognitive problems in patients with epilepsy. For this purpose, differences in glutamate levels between patients with and without side effects should be apparent before start of treatment. Therefore it is important to assess the relationship between glutamate levels and cognition both before and during AED treatment, preferably in longitudinal studies.

The association of cognitive side effects with neuronal activity, should be further assessed and specified using other techniques than MR. *In vitro* studies have been used to assess effects of AED use on neuronal activity and synaptic plasticity,

which can be related to the known side effects of AEDs [12, 13]. Electroencephalography (EEG) can be applied to measure associations of AEDs, cognition and brain activity patterns [14], while positron emission tomography (PET) might give additional information about receptor binding. PET tracers can be applied to measure the affinity of receptors, such as the radiotracer ^{11}C-flumazenil to determine the $GABA_A$ receptor binding [15].

fMRI appears to be the most obvious MR technique to measure neuronal activity, but has some disadvantages when studying AED effects on the brain. fMRI measures the blood oxygenation level dependent (BOLD) activity, which is only indirectly related to brain activity. fMRI is mostly applied to measure task-related activation patterns. Unfortunately, the intrinsic signal responses to the task are little and also the relative change in BOLD signal due to medication is often low, which makes fMRI less suitable to measure global changes in activity [16]. BOLD signals are also sensitive to possible (unwanted) effects of medication on blood flow, and fMRI studies of AED effects should therefore be combined with techniques to measure this blood flow, such as arterial spin labeling (ASL) [16].

Local measurements, distal effects, and brain networks

Current findings

A common theme in this thesis is the connection between local measurements and distal effects, and between local measurements and brain networks. Two questions that frequently arose are:

(i) Does a local finding also reflect distal abnormalities?

(ii) Are characteristics of single regions related to characteristics of other or even global regions?

With MRS, the voxel-of-interest is often placed occipitally, because the highest signal quality can be obtained in that region [17, 18]. This was also the case in Chapter 3, while in this study, the measured glutamate concentrations were associated with information processing speed, a cognitive function that strongly involves the prefrontal cortex [19]. An unanswered question is how these occipital measurements are associated with functions that involve the prefrontal brain regions.

Chapter 6 presents a study that attempts to extend from local MRS measurements to global brain networks. Glutamate and GABA concentrations were measured in 21 distributed brain areas, and connections between these areas were

computed and analyzed. While these neurotransmitter concentrations showed correlations between several distant regions, this correlation was absent between other regions, i.e. we could speak of large-scale spatial organization, so-called networks, for both neurotransmitters. We furthermore measured a higher neurotransmitter connectivity in patients with epilepsy than in healthy participants.

Conceptual background

We assumed that areas that share characteristics, are likely to be connected somehow, a phenomenon known as the 'homophily principle' in social sciences [20]. For two connected areas, the neurotransmitter concentration in one area is positively or negatively associated with the other area [21]. The meaning of these correlations, and mechanisms causing these correlations, are currently unknown, but are likely related to an underlying functional organization of concerted brain activity. While glutamate can be related to excitatory activity, GABA concentrations are related to the fraction of GABAergic neurons [8]. Concentrations of both neurotransmitters have previously been associated with functional connectivity [22]. It is plausible, that areas with shared functionality, show correlated activity, and therefore reveal correlations in neurotransmitter concentrations underlying this activity.

This network concept could explain the association between occipital glutamate concentrations and prefrontal functions, as the glutamate network described in Chapter 6 indeed showed connections between the occipital grey matter and prefrontal white matter. In this scenario, AEDs have a prefrontal effect, and due to that effect the occipital glutamate concentrations are altered, or vice versa: AEDs alter occipital glutamate levels, which affect prefrontal areas, that in its turn affects cognition.

However, as the location of the epileptic focus is not an important factor in the decision of an appropriate AED [9], we can speculate that AEDs have a similar effect on all brain areas. Thus, in our study, an alternative possibility is that the occipital changes in glutamate concentrations reflect global changes.

Methodological considerations

As the study of neurotransmitter networks is a first exploration of this concept, there are several methodological considerations, partly due to scanning difficulties (coarse spatial resolution, long scanning time, scanning artifacts, etc.) and partly as result of the analyses (e.g. how to compensate for differences in grey and white matter content). A major drawback is that at this moment, the networks are formulated at group level instead of individual level. Alternative methods are therefore

required to associate individual clinical measures, such as seizure frequency, drug load or cognitive co-morbidity, to neurotransmitter networks.

As already mentioned, the precise mechanisms underlying the neurotransmitter connections are unknown, and the method provides no actual physical connections, but an implied connection through neuronal correlations of neurotransmitters. Unfortunately, all methods to study *in vivo* brain networks use indirect measures for connections. However, each modality provides unique information about brain functioning, thus the most complete information can be obtained by combining measurements from all different modalities [23].

Clinical implications and future directions

The neurotransmitter connectivity appeared to be higher in patients with epilepsy than healthy participants. Epilepsy is a disease in which large-scale cortical networks are disrupted. Crucial for these networks are inhibitory neurons, synaptic transmission, and neuronal properties [9]. Neurotransmitter networks might be able to give additional information about network dysfunction in epilepsy, as GABA and glutamate concentrations reflect inhibitory neurons and neuronal activity. Future studies might focus on the relation between AED use and neurotransmitter network alterations, because some AEDs alter GABA concentrations (Chapter 2) and cognitive problems were related to glutamate concentrations (Chapter 3). Finally, neurotransmitter network dysfunction might be related to cognitive problems in epilepsy. Studying neurotransmitter networks might therefore help to understand and manipulate the cortical networks in epilepsy (for instance with AEDs), but also in other neurological and psychiatric diseases [24].

Also single-voxel MRS studies might benefit from knowledge of neurotransmitter networks. Neurotransmitter and other neurometabolite concentrations are commonly measured in occipital regions, while the results are associated with cognitive functions [25–27]. Information about the coherence between these neurometabolite concentrations in different brain regions, both in healthy participants and patients, might contribute to the interpretation of these studies.

Validation of MR techniques

Overview thesis

Before novel techniques can be used for clinical or research purposes, validation of these techniques is necessary. For this thesis, we can distinguish two aspects that need validation: firstly, the applied acquisition techniques, i.e. can MRS measure the correct metabolites and concentrations, and can fMRI correctly detect brain

activation? And secondly, the applied analyses methods need validation, especially when discussing brain connectivity.

MR spectroscopy (as applied in Chapter 3) is a commonly used technique to measure *in vivo* neurotransmitter concentrations. The validity of the applied method to measure glutamate concentrations was further assessed in Chapter 5. This chapter presents a study to compare two commonly applied sequences (PRESS and MEGA-PRESS) that enable *in vivo* glutamate concentrations. The results showed that, although both PRESS as MEGA-PRESS had a sufficient accuracy (in phantoms) and *in vivo* reproducibility, the *in vivo* concordance between those sequences was lower than expected. The cause of this low concordance is currently unknown, as both methods could be applied to measure glutamate concentrations. Future studies interested in glutamate measurements, might choose between these two commonly used and easily to implement sequences. While PRESS has a shorter duration and better repeatability than MEGA-PRESS, MEGA-PRESS enables also GABA measurements. Studies interested in both GABA and glutamate, with a limited time, might therefore choose to only perform a MEGA-PRESS scan.

A neurotransmitter network, described in Chapter 6, is a newly introduced concept. As only a correlation on group level is presented, and no individual network measures, no reproducibility measures of network measures could be presented. However, five participants were measured twice, and the results were robust for these within-subjects variations. Measurements of neurotransmitter concentrations showed a moderate to good reproducibility for these participants.

Implications and directions for further studies

At this moment, the study of neurotransmitter networks is still in its infancy. Methodological improvements and necessary validation are required before the measurement of neurotransmitter networks can be applied in daily clinical practice. Therefore, neurotransmitter networks should be further validated in research studies, starting with repeating the current study which larger sample sizes to confirm the current findings.

Further validation of functional brain network analyses was out of the scope of this thesis. However, several arbitrary choices have to be made during this analyses, which can largely affect the result of the study, such as the handling of negative weighted connections [28]. The results described in Chapter 4 differ from those of a previous study, which did find associations between functional brain network organization, AED use (i.e. drug load) and cognitive functioning (IQ) [29]. This latter study included patients with higher drug loads, thus these patients might have suffered from more severe side effects. However, this difference might

also result from differences in analyses methods. Further evaluation of different analyses methods for functional networks is therefore required, and differences in these methods between studies should be considered if comparing these studies.

Conclusion

The studies described in this thesis aimed to identify neuronal substrates of cognitive side effects of AEDs, and to study novel MR techniques that may give new insights on the neurotransmitter function in epilepsy and cognition. The demonstrated associations of glutamate concentrations, AED use, and cognition might be suitable as biomarker for cognitive side effects of AED treatment. Two different sequences to measure these glutamate concentrations (i.e. PRESS and MEGA-PRESS) were studied, and the results showed that both sequences can be employed to measure *in vivo* glutamate concentrations. Finally, the concept of neurotransmitter networks was introduced, which provides a new dimension to further study associations of epilepsy, AED use and cognition.

References

[1] C. Giessing, C. M. Thiel, A. F. Alexander-Bloch, A. X. Patel, and E. T. Bullmore. Human brain functional network changes associated with enhanced and impaired attentional task performance. *J Neurosci*, 33(14):5903–14, 2013.

[2] H. J. Park and K. Friston. Structural and functional brain networks: from connections to cognition. *Science*, 342(6158):1238411, 2013.

[3] M. P. van den Heuvel, C. J. Stam, R. S. Kahn, and H. E. Hulshoff Pol. Efficiency of functional brain networks and intellectual performance. *J Neurosci*, 29(23):7619–24, 2009.

[4] M. A. Rogawski and W. Loscher. The neurobiology of antiepileptic drugs. *Nat Rev Neurosci*, 5 (7):553–64, 2004.

[5] P. Perucca and F. G. Gilliam. Adverse effects of antiepileptic drugs. *The Lancet Neurology*, 11 (9):792–802, 2012.

[6] R. Sankar and G. L. Holmes. Mechanisms of action for the commonly used antiepileptic drugs: Relevance to antiepileptic drug–associated neurobehavioral adverse effects. *Journal of Child Neurology*, 19(1 suppl):S6–S14, 2004.

[7] D. M. IJff and A. P. Aldenkamp. *Comorbidities of treatment with antiepileptic drugs*, pages 424–36. New York: McGraw-Hill Professional, 2012.

[8] C. D. Rae. A guide to the metabolic pathways and function of metabolites observed in human brain 1H magnetic resonance spectra. *Neurochem Res*, 39(1):1–36, 2014.

[9] J. S. Duncan, J. W. Sander, S. M. Sisodiya, and M. C. Walker. Adult epilepsy. *The Lancet*, 367 (9516):1087–1100, 2006.

[10] C. E. Elger, C. Helmstaedter, and M. Kurthen. Chronic epilepsy and cognition. *The Lancet Neurology*, 3(11):663–672, 2004.

[11] R. S. Fisher, B. G. Vickrey, P. Gibson, B. Hermann, P. Penovich, A. Scherer, and S. Walker. The impact of epilepsy from the patient's perspective II: views about therapy and health care. *Epilepsy Research*, 41(1):53–62, 2000.

[12] C. Sgobio, V. Ghiglieri, C. Costa, V. Bagetta, S. Siliquini, I. Barone, M. Di Filippo, F. Gardoni, E. D. Gundelfinger, M. Di Luca, B. Picconi, and P. Calabresi. Hippocampal synaptic plasticity,

memory, and epilepsy: effects of long-term valproic acid treatment. *Biol Psychiatry*, 67(6):567–74, 2010.

[13] P. J. West, G. W. Saunders, G. J. Remigio, K. S. Wilcox, and H. S. White. Antiseizure drugs differentially modulate theta-burst induced long-term potentiation in C57BL/6 mice. *Epilepsia*, 55(2):214–223, 2014.

[14] M. C. Salinsky, L. M. Binder, B. S. Oken, D. Storzbach, C. R. Aron, and C. B. Dodrill. Effects of gabapentin and carbamazepine on the EEG and cognition in healthy volunteers. *Epilepsia*, 43(5): 482–490, 2002.

[15] O. M. Weber, A. Verhagen, C. O. Duc, D. Meier, K. L. Leenders, and P. Boesiger. Effects of vigabatrin intake on brain GABA activity as monitored by spectrally edited magnetic resonance spectroscopy and positron emission tomography. *Magnetic resonance imaging*, 17(3):417–425, 1999.

[16] B. Wandschneider and M. J. Koepp. Pharmaco fMRI: Determining the functional anatomy of the effects of medication. *Neuroimage Clin*, 12:691–697, 2016.

[17] C. J. Evans, N. A. Puts, S. E. Robson, F. Boy, D. J. McGonigle, P. Sumner, K. D. Singh, and R. A. Edden. Subtraction artifacts and frequency (mis-)alignment in J-difference GABA editing. *J Magn Reson Imaging*, 38(4):970–5, 2013.

[18] N. A. Puts and R. A. Edden. In vivo magnetic resonance spectroscopy of GABA: a methodological review. *Prog Nucl Magn Reson Spectrosc*, 60:29–41, 2012.

[19] R. Cabeza and L. Nyberg. Imaging cognition II: An empirical review of 275 PET and fMRI studies. *Journal of cognitive neuroscience*, 12(1):1–47, 2000.

[20] Ö. Şimşek and D. Jensen. Navigating networks by using homophily and degree. *Proc Natl Acad Sci U S A*, 105(35):12758–62, 2008.

[21] J. W. Pan, D. D. Spencer, R. Kuzniecky, R. B. Duckrow, H. Hetherington, and S. S. Spencer. Metabolic networks in epilepsy by MR spectroscopic imaging. *Acta Neurol Scand*, 126(6):411–20, 2012.

[22] N. W. Duncan, C. Wiebking, and G. Northoff. Associations of regional GABA and glutamate with intrinsic and extrinsic neural activity in humans – a review of multimodal imaging studies. *Neurosci Biobehav Rev*, 47C:36–52, 2014.

[23] A. Fornito, A. Zalesky, and M. Breakspear. Graph analysis of the human connectome: promise, progress, and pitfalls. *NeuroImage*, 80:426–44, 2013.

[24] S. S. Spencer. Neural networks in human epilepsy: evidence of and implications for treatment. *Epilepsia*, 43(3):219–227, 2002.

[25] A. J. Ross and P. S. Sachdev. Magnetic resonance spectroscopy in cognitive research. *Brain Res Rev*, 44(2-3):83–102, 2004.

[26] K. Sandberg, J. U. Blicher, M. Y. Dong, G. Rees, J. Near, and R. Kanai. Occipital GABA correlates with cognitive failures in daily life. *Neuroimage*, 87:55–60, 2014.

[27] F. C. van Bussel, W. H. Backes, P. A. Hofman, N. A. Puts, R. A. E. Edden, M. P. van Boxtel, M. T. Schram, C. D. Stehouwer, J. E. Wildberger, and J. F. A. Jansen. Increased GABA concentrations in type 2 diabetes mellitus are related to lower cognitive functioning. *Medicine (Baltimore)*, 95 (36):e4803, 2016.

[28] A. Weissenbacher, C. Kasess, F. Gerstl, R. Lanzenberger, E. Moser, and C. Windischberger. Correlations and anticorrelations in resting-state functional connectivity MRI: a quantitative comparison of preprocessing strategies. *NeuroImage*, 47(4):1408–16, 2009.

[29] M. C. G. Vlooswijk, M. J. Vaessen, J. F. A. Jansen, M. C. F. T. M. de Krom, H. J. M. Majoie, P. A. M. Hofman, A. P. Aldenkamp, and W. H. Backes. Loss of network efficiency associated with cognitive decline in chronic epilepsy. *Neurology*, 77(10):938–944, 2011.

Addendum

Summary

Epilepsy is a neurological disease which is characterized by unprovoked recurrent seizures, during which the brain shows abnormal and excessive neuronal activity. The majority of the patients use antiepileptic drugs (AEDs) to suppress epileptic seizures. Unfortunately, these AEDs also induce adverse effects, such as cognitive problems.

The brain can be considered as a network, encompassing neurons which are connected via synapses. It is assumed that the integrity of brain networks is important for cognitive functioning, and that this integrity is affected in neurological diseases as epilepsy. Different magnetic resonance (MR) techniques can be employed to assess different aspects of brain connectivity: functional MR imaging (fMRI) enables the assessment of functional brain networks, i.e. the framework of all, functionally connected brain areas, while MR spectroscopy (MRS) enables measurements of the neurotransmitters GABA and glutamate, the most abundant inhibitory and excitatory neurotransmitters in the brain, respectively. The aim of this thesis, as described in **Chapter 1**, was to further assess the associations between brain connectivity and cognitive problems in epilepsy.

In **Chapter 2**, an overview is given of previous MR studies that assessed effects of AEDs on the brain. MRS studies showed that AEDs with GABAergic mechanisms of action (such as topiramate and vigabatrin) increase the cerebral GABA concentrations. Effects on glutamate concentrations were thus far less frequently assessed and therefore not known conclusively. **Chapter 2** also discussed fMRI studies of AED effects. Although at neuronal level, AEDs aim to suppress (hyper) excitability, fMRI studies showed that at the brain level, brain activity can be decreased, but also unaffected or even increased. The effects of AEDs on brain activity differed between AED-type and brain regions.

Chapter 3 and **4** describe two studies that assessed associations between AEDs, cognitive problems, and *in vivo* neurotransmitter levels or brain network organization, respectively. For these studies, three groups of patients on chronic AED treatment were included: One group using AEDs with a low risk for cognitive side effects (lamotrigine or levetiracetam, n=16), one group using AEDs with an intermediate risk for cognitive side effects (carbamazepine, oxcarbazepine, phenytoin or valproate, n=34), and a group using AEDs with a high risk for cognitive side effects (topiramate, n=5). All patients underwent cognitive testing and MR scan-

ning. The Visual Computerized Searching Task (CVST) was applied to measure complex information processing speed, which is often affected by AED use. MRS was performed to measure *in vivo* neurotransmitter levels, and the brain network organization was measured using resting-state fMRI.

The associations between AEDs, cognitive problems, and neurotransmitter levels are described in **Chapter 3**. Patients with decreased processing speeds showed lower glutamate concentrations than patients with a normal processing speed. Furthermore, the glutamate concentrations were also significantly lower in the high and intermediate-risk category than in the low-risk category. No significant associations were found between GABA and information processing speed or risk category. Based on this study, we concluded that lower glutamate concentrations are related to AED use and slow down central information processing in patients with epilepsy.

Chapter 4 describes associations between AEDs, cognition, and functional brain network measures. Two measures were described: the clustering coefficient and the global efficiency, which are measures for functional segregation and integration, respectively. No associations were observed between information processing speed and these brain network measures, and only the high-risk category showed an increased global efficiency, which might be due to compensatory mechanisms. Alterations in functional brain network organization may be only subtle in the patients studied and may become measureable in patients with more severe cognitive side effects.

For further evaluation, MR techniques were studied that may give new insights in epilepsy and cognition. **Chapter 5** presents a study that evaluated different MRS methods to measure glutamate levels. Although not specifically designed for this purpose, PRESS and MEGA-PRESS sequences are both often used to measure glutamate. PRESS is a commonly applied method to measure neurometabolites, but is not able to measure GABA concentrations, while MEGA-PRESS allows for quantification of GABA. **Chapter 5** compares the accuracy, repeatability, and concordance of these sequences. Phantom experiments showed a good accuracy for both sequences. The repeatability was tested in five healthy participants and was sufficient in both sequences, but was better in PRESS than in MEGA-PRESS. However, the concordance between the sequences was only moderate (r=0.4), which might be due to macromolecule contamination in the PRESS or MEGA-PRESS spectra. Based on this study, we concluded that MEGA-PRESS can be used to combine GABA and glutamate measurements, albeit at a cost of lower repeatability compared with PRESS.

Chapter 6 describes a new method to assess brain networks based on metabolic information, in particular glutamate, GABA, and N-acetylaspartate measurements.

Many clinical neuroimaging studies are focused on altered structural or functional brain connectivity. However, these studies cannot provide direct information on the defective neurons or the linked neurotransmitter disbalance, which underlies abnormal neuronal activity. Therefore, the concept of 'neurotransmitter networks' was introduced. Areas were considered connected if the Pearson's correlation coefficient was significant between those areas, across the participants.

This concept was tested in fifteen healthy participants and ten patients with cryptogenic localization related epilepsy. In the healthy participants, 21%, 19%, and 26% of the possible connected areas showed a significant correlation for glutamate, GABA, and NAA, respectively. These connections can be conceptualized as 'metabolic brain networks'. When comparing networks from patients with epilepsy to those from healthy participants, an increased glutamate and GABA connectivity was found in the epilepsy group. We concluded that these neurotransmitter networks, and the increased neurotransmitter connectivity in patients with epilepsy, should be further explored to increase insights into epilepsy.

This thesis is finished with a general discussion in **Chapter 7**. This chapter provides a general overview of the main findings in this thesis, discusses these findings in a broader perspective and provides recommendations for further research.

Samenvatting

Epilepsie is een neurologische aandoening die gekarakteriseerd wordt door niet-uitgelokte, herhaald optredende aanvallen. Tijdens deze aanvallen vertoont het brein abnormale excessieve neuronale activiteit. De meerderheid van de patiënten gebruikt anti-epileptica om epileptische aanvallen te onderdrukken. Deze medicatie zorgt echter ook voor bijwerkingen, waaronder cognitieve problemen.

Het brein kan worden gezien als een netwerk bestaande uit neuronen die verbonden zijn via synapsen. Er wordt aangenomen dat de integriteit van dit hersennetwerk belangrijk is voor het cognitief functioneren, maar afwijkend is als sprake is van epilepsie. Verschillende magnetische resonantietechnieken zijn beschikbaar om de hersennetwerken te onderzoeken: functionele magnetische resonantie imaging (fMRI) maakt het mogelijk om functionele hersennetwerken te onderzoeken, terwijl met magnetische resonantie spectroscopie (MRS) de concentraties van de meest voorkomende activerende en remmende neurotransmitters in het brein (glutamaat en GABA) gemeten kunnen worden. Het doel van dit proefschrift, zoals beschreven in **hoofdstuk 1**, was om de samenhang tussen hersenconnectiviteit en cognitieve problemen als gevolg van epilepsie verder te onderzoeken.

Hoofdstuk 2 geeft een overzicht van eerdere MR studies die effecten van anti-epileptica op het brein hebben onderzocht. MRS studies hebben aangetoond dat anti-epileptica met GABAerge werkingsmechanismen (zoals topiramaat en vigabatrine) de GABA-concentraties in het brein verhogen. Het effect op glutamaatconcentraties is minder vaak onderzocht en daardoor niet duidelijk. Ook fMRI studies naar effecten van anti-epileptica op het brein worden besproken in **hoofdstuk 2**. Op celniveau hebben anti-epileptica als doel de (hyper)excitabiliteit te onderdrukken. Echter, fMRI studies hebben aangetoond dat op breinniveau, anti-epileptica hersenactiviteit kunnen onderdrukken, maar ook onaangedaan laten of zelfs stimuleren. De precieze effecten van anti-epileptica op de hersenactiviteit verschilden per type anti-epilepticum en hersengebied.

Hoofdstuk 3 en **4** beschrijven twee studies waarin de samenhang tussen langdurig gebruik van anti-epileptica, cognitieve problemen, en *in vivo* neurotransmitterconcentraties op hersennetwerkorganisatie onderzocht is. Voor deze studies zijn drie groepen epilepsiepatiënten geïncludeerd: De eerste groep bestond uit patiënten die anti-epileptica gebruiken met een laag risico op bijwerkingen (lamotrigine of levetiracetam, n = 16). De tweede groep bestond uit patiënten die anti-epileptica

gebruiken met een gemiddeld risico op cognitieve bijwerkingen (carbamazepine, fenytoïne, oxcarbazepine of valproaat, n=34), terwijl de derde groep bestond uit patiënten die anti-epileptica gebruiken met een hoog risico op cognitieve bijwerkingen (topiramaat, n=5). Alle patiënten ondergingen cognitieve testen en een MR scan. De Computerized Visual Searching Task (CVST) is gebruikt om de verwerkingssnelheid van complexe informatie te meten, een cognitieve functie die vaak is aangedaan door anti-epileptica. Neurotransmitterconcentraties zijn gemeten met behulp van MRS, en de organisatie van de hersennetwerken in rust zijn met behulp van fMRI gemeten.

De samenhang tussen anti-epileptica, cognitieve problemen, en neurotransmitterconcentraties wordt beschreven in **hoofdstuk 3**. De glutamaatconcentratie bleek positief gecorreleerd met de informatieverwerkingssnelheid. Met andere woorden, patiënten met een lagere informatiesnelheid, hadden ook een lagere glutamaatconcentratie. Verder was deze concentratie significant lager in de patiënten die medicatie met een hoog of gemiddeld risico op bijwerkingen gebruikten, dan in de patiënten die medicatie met een laag risico op bijwerkingen gebruikten. Tussen de GABA-concentratie en informatieverwerkingssnelheid, of tussen de GABA-concentratie en risicocategorie, waren geen significante associaties. Gebaseerd op deze resultaten concludeerden we dat lagere glutamaatconcentraties gerelateerd zijn aan het gebruik van anti-epileptica en een vertraagde informatieverwerking in epilepsiepatiënten.

Hoofdstuk 4 beschrijft de samenhang tussen anti-epileptica, cognitie, en breinnetwerkmaten. Twee verschillende maten werden beschreven: de clustercoëfficiënt en de globale efficiëntie, welke maten zijn voor de respectievelijke functionele segregatie en integratie. Er werden geen associaties geobserveerd tussen informatieverwerkingssnelheid en deze netwerkmaten. Wel liet de hoog-risicogroep een significant hogere globale efficiëntie zien dan de laag- en gemiddeld-risicogroepen, wat mogelijk het gevolg is van een compensatiemechanisme. Wijzigingen in functionele hersennetwerkorganisatie zijn mogelijk subtiel en alleen meetbaar in patiënten met ernstigere cognitieve bijwerkingen.

Het tweede deel van dit proefschrift bevat studies waarin MR technieken zijn onderzocht die mogelijk nieuwe inzichten in epilepsie en cognitie kunnen geven. **Hoofdstuk 5** beschrijft een studie waarin verschillende MRS methoden om glutamaatconcentraties te meten werden vergeleken. De sequenties PRESS en MEGA-PRESS worden beide vaak gebruikt om glutamaatconcentraties te meten, hoewel ze hier niet speciaal ontworpen voor zijn: PRESS wordt veel gebruikt om verschillende neurometabolietconcentraties te meten, maar kan geen GABA-concentraties meten. MEGA-PRESS is juist ontworpen om deze GABA-concentraties te meten. **Hoofdstuk 5** vergelijkt de accuraatheid, reproduceerbaarheid, en overeen-

stemming van glutamaatmetingen met deze sequenties. Fantoommetingen lieten een goede accuraatheid voor beide sequenties zien. De reproduceerbaarheid is getest in vijf gezonde proefpersonen, en bleek toereikend voor beide sequenties, hoewel beter voor PRESS dan MEGA-PRESS. De overeenkomst tussen PRESS en MEGA-PRESS was echter matig (r=0.4), wat mogelijk verklaard kan worden door de aanwezigheid van macromoleculen in het PRESS of MEGA-PRESS spectrum. Gebaseerd op deze studie concludeerden we dat MEGA-PRESS inderdaad gebruikt kan worden om GABA en glutamaatmetingen te combineren, hoewel met een lagere reproduceerbaarheid dan wanneer PRESS gebruikt zou kunnen worden.

Hoofdstuk 6 beschrijft een nieuwe methode om hersennetwerken te onderzoeken, gebaseerd op metabole informatie verkregen uit glutamaat, GABA, en N-acetylaspartaat metingen. Klinische studies bestuderen voornamelijk structurele en functionele breinnetwerken. Echter, hiermee wordt geen directe informatie over neuronale integriteit of neurotransmitterbalans verkregen, wat de oorzaak is van abnormale neuronale activiteit in epilepsie. Daarom introduceerden wij het 'neurotransmitternetwerk' concept. Gebieden in de hersenen werden verondersteld verbonden te zijn, als de correlatie tussen deze gebieden (gezien over de proefpersonen) significant was.

Dit concept is getest in vijftien gezonde proefpersonen en tien patiënten met cryptogene, lokalisatie-gebonden epilepsie. In de gezonde proefpersonen liet 21%, 19% en 26% van de mogelijke verbindingen een significante correlatie voor respectievelijk glutamaat, GABA en NAA zien. Deze verbindingen kunnen worden gezien als een 'metabool breinnetwerk'. Zowel de glutamaat, als de GABA netwerken van de epilepsiepatiënten hadden een hogere connectiviteit dan die van de gezonde proefpersonen. We concludeerden dat deze neurotransmitternetwerken en verhoogde neurotransmitterconnectiviteit in epilepsiepatiënten verder onderzocht zouden moeten worden om de kennis over epilepsie te vergroten.

Het proefschrift wordt afgesloten met een algemene discussie in **hoofdstuk 7**. Dit hoofdstuk geeft een overzicht van de belangrijkste bevindingen in dit proefschrift, bediscussieert de bevindingen in een breder geheel en geeft aanbevelingen voor vervolgonderzoek.

Valorization

Relevance

Thousands of MR studies have been performed to investigate functional brain networks and neurotransmitter concentrations. These studies are primarily performed in the fields of neurology, neurosciences and psychology. Two different aims can be distinguished in these studies: firstly, to increase the understanding of healthy or diseased brain functioning; and secondly, to detect biomarkers that might be used for diagnosis, to predict disease progression or treatment effects [1–3].

In this thesis, we focused on brain connectivity in epilepsy, and its relation to cognition. In the Netherlands, there are approximately 120,000 patients with epilepsy, and six-thousand new patients are diagnosed every year [4]. Epilepsy is often accompanied with cognitive problems, such as impaired memory, concentration, or slowed information processing speed [5]. These cognitive problems may be caused by the epilepsy itself, the underlying etiology, or as side effect of antiepileptic drug (AED) treatment. At this moment, neurologists have no objective means to predict who will develop adverse side effects or not.

We mainly focused on the effects AED treatment in this thesis. Cognitive side effects are among the least tolerated side effects for patients with epilepsy [6], and are an important reason to halt or change the AED treatment [7]. Besides a large burden for the patients, cognitive side effects are also accompanied with economic costs, including increased health care costs, productivity losses, and patients and family costs. The total societal costs of cognitive side effects are estimated to be around 7,000 € per patient per year in the Netherlands [8]. Better understanding of the relation between epilepsy, AED treatment, and cognition might help during clinical decision-making and ultimately improve treatment of patients with epilepsy.

Main findings

In the first part of this thesis, clinical studies are presented that assessed associations between AED use, cognition, and MR markers. We observed associations between decreased information processing speed and lower glutamate concentrations. These glutamate concentrations were also associated with AED use. The second part of this thesis described methodological studies to neurotransmitter

measurements. We compared two methods to measure *in vivo* glutamate concentrations using MR spectroscopy: PRESS and MEGA-PRESS. This study showed a good accuracy for both methods, although the repeatability was better in PRESS than MEGA-PRESS. Finally, we developed a method to assess 'neurotransmitter networks', and showed an increased glutamate and GABA connectivity in patients with epilepsy.

Target groups

First of all, the findings of this study are relevant for patients with epilepsy, as the findings might, in future, improve pharmacological treatment in epilepsy. This is also of interest for their neurologist, who might be better able to select proper treatment, and the pharmaceutical industry. Pharmaceutical applications of MRI are starting to develop, and MR markers might be beneficial to capture cognitive side effects or treatment efficacy in an early state, or to explore proper dose ranges [9].

The methodological part is mostly relevant for other researchers in the field of neurology, neurosciences and psychology, as it might aid new studies of the involvement of neurotransmitter levels in different neurological, psychological and psychiatric diseases. Therefore, other patients suffering from these diseases might benefit from these studies as well.

Innovation and future directions

The studies presented in this thesis should be considered as explorations on the interplay of MR methods and epilepsy, AED treatment, and cognition. While advanced MR imaging of epilepsy and its consequences is an emerging field, MR studies to study AED effects are relatively scarce and to our knowledge, the studies in this thesis are the first that associate AED use and cognitive functioning to respectively neurotransmitter levels and functional brain organization. The observed associations between glutamate levels and cognitive function might be useful as biomarker to predict which patient will suffer from cognitive side effects, and who will not. However, it is currently not known whether the differences in glutamate levels precede the cognitive problems or coincide with these problems. Therefore, longitudinal studies to this topic are required.

The main objective of the comparison between PRESS and MEGA-PRESS was to aid future clinical studies aiming to measure glutamate concentrations. In our study, we applied both PRESS, to measure glutamate concentrations, and MEGA-PRESS, to measure GABA concentrations. We showed that the glutamate

concentration could also be measured with MEGA-PRESS; comparable studies might therefore choose to only apply a MEGA-PRESS scan, and thereby shortening the scanning time.

Neurotransmitter networks, described in Chapter 6, are a newly introduced concept and might provide new possibilities to study brain function. Future studies should aim at a further evaluation of this concept. Optimization is still possible at the acquisition and the analysis stage of the data. Furthermore, the concept should be tested in larger groups and in different populations, such as children or elderly, or in different brain diseases.

References

[1] G. Öz, J. R. Alger, P. B. Barker, R. Bartha, A. Bizzi, C. Boesch, P. J. Bolan, K. M. Brindle, C. Cudalbu, and A. Dinçer. Clinical proton MR spectroscopy in central nervous system disorders. *Radiology*, 270(3):658–679, 2014.

[2] A. J. Ross and P. S. Sachdev. Magnetic resonance spectroscopy in cognitive research. *Brain Res Rev*, 44(2-3):83–102, 2004.

[3] C. Giessing and C. M. Thiel. Pro-cognitive drug effects modulate functional brain network organization. *Front Behav Neurosci*, 6:53, 2012.

[4] Epilepsiefonds. Bij wie en op welke leeftijd. https://www.epilepsie.nl/over-epilepsie/pagina/224-1/bij-wie-en-op-welke-leeftijd/.

[5] R. S. Fisher, B. G. Vickrey, P. Gibson, B. Hermann, P. Penovich, A. Scherer, and S. Walker. The impact of epilepsy from the patient's perspective I. descriptions and subjective perceptions. *Epilepsy research*, 41(1):39–51, 2000.

[6] J.-A. Witt, C. E. Elger, and C. Helmstaedter. Which drug-induced side effects would be tolerated in the prospect of seizure control? *Epilepsy & Behavior*, 29(1):141–143, 2013.

[7] H. P. Bootsma, L. Ricker, Y. A. Hekster, J. Hulsman, D. Lambrechts, M. Majoie, A. Schellekens, M. de Krom, and A. P. Aldenkamp. The impact of side effects on long-term retention in three new antiepileptic drugs. *Seizure*, 18(5):327–331, 2009.

[8] R. J. A. de Kinderen, S. M. A. A. Evers, R. Rinkens, D. Postulart, C. I. Vader, M. H. J. M. Majoie, and A. P. Aldenkamp. Side-effects of antiepileptic drugs: the economic burden. *Seizure*, 23(3):184–190, 2014.

[9] B. Wandschneider and M. J. Koepp. Pharmaco fMRI: Determining the functional anatomy of the effects of medication. *Neuroimage Clin*, 12:691–697, 2016.

Dankwoord

Zoals gebruikelijk wil ik in dit laatste hoofdstuk iedereen die heeft bijgedragen aan dit proefschrift bedanken. Sommigen hebben hele concrete bijdragen geleverd. Anderen stonden meer op de achtergrond, maar ook zonder hen had ik dit niet kunnen afronden.

Allereerst wil ik graag mijn promotieteam bedanken. Walter Backes, Bert Aldenkamp en Jaap Jansen, bedankt dat jullie mij de mogelijkheid hebben geboden voor dit promotieonderzoek, en voor jullie altijd betrokken en toegankelijke begeleiding. Dit heb ik als zeer prettig ervaren.

Walter, jouw feedback kwam meestal erg snel, soms zelfs sneller dan ik eigenlijk zou willen. Wat een luxe! Tegelijkertijd ben je altijd kritisch, maar ook erg betrokken bij jouw promovendi, wat volgens mij een goede promotor kenmerkt. Bert, jouw bijdrage was wat meer op de achtergrond, maar jouw ideeën over en de feedback op met name de klinische onderdelen zijn een grote bijdrage geweest aan dit proefschrift. Jaap, jouw deur stond altijd open en dit heb ik zeer op prijs gesteld. Je weet een kritische blik te combineren met een pragmatische aanpak. Dit laatste was voor mij soms wat wennen, maar daarmee ook erg leerzaam. Bedankt voor deze samenwerking!

Verder ben ik dank verschuldigd aan al mijn co-auteurs. Allereerst mijn dank aan Dominique: Wat heb jij hard gewerkt aan de AED studie! Ik heb bewondering voor jouw niet-aflatende energie en wil je veel succes wensen met jouw carrière. Rob Rouhl en Marielle Vlooswijk: Tien geschikte proefpersonen vinden bleek heel lastig te zijn, maar het is op het nippertje toch gelukt! Bedankt daarvoor, en zeker ook voor jullie kritische inbreng tijdens het onderzoek en schrijven van het artikel. Ook Paul Hofman, Richard Lazeron, Anton de Louw, Frank van Bussel, Tom Scheenen en Dennis Klomp dank ik voor hun inbreng. Finally, I would like to thank Richard Edden, Nick Puts, and Desmond Tse for their contribution as co-author.

Ook anderen hebben een essentiële bijdrage geleverd aan mijn onderzoek: Allereerst natuurlijk alle proefpersonen die hieraan meegewerkt hebben. Verder wil ik Remco Berting bedanken voor het scannen bij Kempenhaeghe, en Esther Steijvers en Lotty Huijboom voor het scannen bij Scannexus. Bedankt voor alle gezellige uurtjes achter de scanner! Voor het maken van de fantomen moet ik Ingrid Dijkgraaf en haar collega's bedanken, voor het beschikbaar stellen van hun labfaciliteiten.

Als laatste ben ik Jos en Marc veel dank verschuldigd voor alle computerfaciliteiten binnen de afdeling Radiologie, zonder welke ik niet alle analyses had kunnen uitvoeren.

Daarnaast wil ik mijn kamergenoten en collega's bedanken: René, voor het helpen aan het begin van mijn promotie; Frank, Harm en May voor al jullie advies én de gezellige uurtjes; en Gerald, Laura, Lisanne en Joost, bedankt voor de gezelligheid, en succes tijdens jullie verdere promoties/postdoc-periode! Als laatste wil ik al mijn overige collega's bij de afdeling Radiologie en bij Kempenhaeghe bedanken voor hun gezelligheid tijdens de lunch, borrels en de Kempenhaeghe-etentjes.

Ook een kort woord van dank aan mijn paranimfen May en Ingeborg. Waar ik bijna mijn gehele promotietijd een kamer met May heb gedeeld, heb ik tijdens vrijwel mijn hele studie samen met Ingeborg in één huis gewoond. Wat heb ik veel gezelligheid, maar ook frustraties met jullie kunnen delen: niet alleen over onze promoties, maar ook over alle andere dingen in ons leven. Fijn dat jullie naast mij willen staan tijdens mijn verdediging!

Mijn laatste woorden zijn voor de mensen die het dichtst bij mij staan. Papa en mama, hoewel jullie hadden gehoopt dat ik na mijn studie weer wat dichterbij zou komen wonen, hebben jullie mij altijd gesteund en aangemoedigd om te gaan promoveren in Maastricht. Bedankt dat jullie er altijd voor mij zijn. Lieve Fokke, we hadden nog maar net een relatie toen ik solliciteerde voor deze promotie. Mijn keuze om naar Maastricht te gaan heeft het ook voor jou niet makkelijker gemaakt, maar we hebben ons samen door de vele treinreizen heen geslagen. En hoewel voor ons beiden het 'Hora est' nog moet komen, durf ik al wel te stellen dat het ons gelukt is! Dank je wel!

Curriculum Vitae

Tamar Marije van Veenendaal was born in Nijkerk at March 6th, 1988. In 2000, she started secondary school at the Christelijk College Nassau-Veluwe in Harderwijk. After her graduation in 2006, she moved to Enschede to study Biomedical Engineering at Twente University, with as specialization Human Function Technology. During her master she did an internship at Aalborg University, Denmark in which she performed experiments to assess heat hyperalgesia in human participants. She received her master's degree in 2012, after finishing her thesis focused on the computational modeling of neuronal networks.

In 2013, she went to Maastricht University for her PhD research, which is described in this thesis. This research was performed within the School for Mental Health and Neuroscience (MHeNS) under supervision of prof. dr. ir. W.H. Backes, prof. dr. A.P. Aldenkamp, en dr. J.F.A. Jansen and in collaboration with the epilepsy center Kempenhaeghe. After her PhD, she will start working as software engineer for the High Tech division of Sogeti Nederland B.V.

List of publications

This thesis

T.M. van Veenendaal, D.M. IJff, A.P. Aldenkamp, P.A.M. Hofman, M.C.G. Vlooswijk, R.P.W. Rouhl, A.J. de Louw, W.H. Backes, J.F.A. Jansen, "Metabolic and functional MR biomarkers of antiepileptic drug effectiveness: a review", *Neuroscience & Biobehavioral Reviews*, 59:92–99, 2015.

T.M. van Veenendaal, D.M. IJff, A.P. Aldenkamp, R.H.C. Lazeron, N.A.J. Puts, R.A.E. Edden, P.A.M. Hofman, A.J. de Louw, W.H. Backes, J.F.A. Jansen, "Glutamate concentrations vary with antiepileptic drug use and mental slowing", *Epilepsy & Behavior*, 64:200–205, 2016.

T.M. van Veenendaal, D.M. IJff, A.P. Aldenkamp, R.H.C. Lazeron, P.A.M. Hofman, A.J. de Louw, W.H. Backes, J.F.A. Jansen, "Chronic antiepileptic drug use and functional network efficiency: A functional magnetic resonance imaging study", *World Journal of Radiology, In press.*

T.M. van Veenendaal, W.H. Backes, F.C.G. van Bussel, R.A.E. Edden, N.A.J. Puts, A.P. Aldenkamp, J.F.A. Jansen "Glutamate quantification by PRESS or MEGA-PRESS: accuracy, repeatability, and concordance", *Submitted.*

T.M. van Veenendaal, W.H. Backes, D.H.Y. Tse, T.W.J. Scheenen, D.W. Klomp, P.A.M. Hofman, R.P.W. Rouhl, M.C.G. Vlooswijk, A.P. Aldenkamp, J.F.A. Jansen, "High field imaging of large-scale neurotransmitter networks: proof of concept and initial application to epilepsy", *Submitted.*

Other publications

J. le Feber, T. Witteveen, T.M. van Veenendaal, J. Dijkstra, "Repeated stimulation of cultured networks of rat cortical neurons induces parallel memory traces", *Learning & memory*, 22(12):594–603, 2015.

D.M. IJff, T.M. van Veenendaal, H.J.M. Majoie, A.J.A. de Louw, J.F.A. Jansen, A.P. Aldenkamp, "Cognitive effects of lacosamide as adjunctive therapy in refractory epilepsy", *Acta Neurologica Scandinavica*, 131(6):347–354, 2015.

F.C.G. van Bussel, W.H. Backes, T.M. van Veenendaal, P.A.M. Hofman, M.P.J. van Boxtel, M.T. Schram, S.J.S. Sep, P.C. Dagnelie, N. Schaper, C.D.A. Stehouwer, J.E. Wildberger, J.F.A. Jansen, "Functional brain networks are altered in type 2 diabetes and prediabetes: signs for compensation of cognitive decrements? The Maastricht study", *Diabetes*, 65(8):2404–2413, 2016.

D.M. IJff, T.M. van Veenendaal, M.H. Debeij-van Hall, J.F.A. Jansen, A.J.A. de Louw, M.H.J.M. Majoie, A.P. Aldenkamp, "The Cognitive Profile of Ethosuximide in Children", *Pediatric Drugs*, 18(5):379–385, 2016.

G.S. Drenthen, E.M. Barendse, A.P. Aldenkamp, T.M. van Veenendaal, N.A.J. Puts, R.A.E. Edden, S. Zinger, G. Thoonen, M.P.H. Hendriks, R.P.C. Kessels, J.F.A. Jansen, "Altered neurotransmitter metabolism in adolescents with high-functioning autism", *Psychiatry Research: Neuroimaging*, 256:44–49, 2016.