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Proton MRS Studies in Ageing: investigating relaxation, concentration, and correlation with resting-state activity in the PCC

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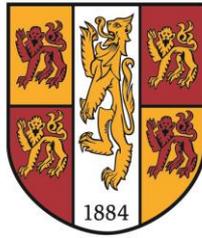*Awarding institution:*
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Proton MRS Studies in Ageing: investigating relaxation, concentration, and correlation with resting-state activity in the PCC

Karolina W. Rusiak

Thesis submitted to the School of Psychology, Bangor University,
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July 2016

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I delight in what I fear.

(Shirley Jackson)

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List of abbreviations

^{1}H	hydrogen nuclei, proton
^{1}H-MRS	proton magnetic resonance spectroscopy
ACC	anterior cingulate cortex
B_0	external magnetic field
B_1	oscillating magnetic field
BBB	blood brain barrier
BF	bayesian factor
BOLD	blood-oxygen-level dependent
CHESS	chemical shift selective
Cho	the sum of choline, phosphocholine and glycero-phosphocholine
Cr	the sum of creatine and phosphocreatine
CSF	cerebrospinal fluid
DMN	default mode network
fMRI	functional magnetic resonance imaging
GABA	γ-aminobutyric acid
GABA+	γ-aminobutyric acid and macromolecules
Gln	glutamine
Glu	glutamate
Glx	glutamate + glutamine
GM	grey matter
M	resting magnetization
MEGA-PRESS	mescher-garwood point-resolved spectroscopy
MI	myo-inositol
mM	millimolar
MM	macromolecules
MMSE	mini mental state examination
MRI	magnetic resonance imaging
ms	milliseconds
NAA	n-acetylaspartate
NMR	nuclear magnetic resonance
PCC	posterior cingulate cortex
PRESS	point resolved spectroscopy
ppm	parts per million

RF	radiofrequency
ROI	region of interest
SNR	signal-to-noise ratio
T	tesla
T_1	spin-lattice (longitudinal) relaxation time of nucleus
T_2	spin-spin (transverse) relaxation time of nucleus
T_2^*	apparent transverse relaxation
$T_2^\dagger$	represents apparent T_2 findings in Chapter 4
TE	echo time
TM	mixing time
TR	repetition time
VOI	voxel of interest
WM	white matter

Summary

The human brain undergoes changes over its lifespan, which may appear in the form of cognitive decline or disease. As such, it is necessary to investigate the ageing process at the neurochemical level as normal and pathological processes may overlap. The thesis examines metabolite transverse (T_2) relaxation times as well as metabolite concentrations in relation to cognitive performance and functional connectivity across age. A systematic review of the T_2 relaxation literature offers consensus T_2 relaxation values for N-Acetyl Aspartate (NAA), creatine, and choline across tissue content at 1.5 and 3 Tesla for accurate quantification of metabolite concentration levels. Building on these findings, the first empirical study investigates T_2 relaxation values across age in healthy younger and typically ageing older adults. The results suggest a significant difference in NAA apparent $T_2^{\dagger}$ relaxation values between the younger and older cohort. The findings from the systematic review and first empirical study are used for accurate quantification of metabolite concentration levels in the second and third empirical studies. The second empirical study examines the relation of the major excitatory (glutamate) and inhibitory (GABA) neurotransmitters to cognitive performance across age. The outcome suggests age and reduced glutamate concentration levels to be predictors of cognitive performance on selective cognitive tests. In the third empirical study, glutamate and GABA concentrations are assessed in relation to functional connectivity between the posterior cingulate cortex and hippocampus, brain regions that are affected in mild cognitive impairment and Alzheimer's disease. The findings, considered with caution, suggest that higher glutamate concentrations are associated with increased functional connectivity between posterior cingulate cortex and hippocampus. Taken together these studies shed light into the ageing process by characterising neurochemical mechanisms in relation to cognitive performance and functional connectivity. The utilization of proton magnetic resonance spectroscopy as well as functional magnetic resonance imaging can provide underpinnings of healthy ageing along with pathologies.

Chapter 1

Introduction

1.1. Introduction

As ageing is a heterogeneous process, the thesis aim is to extend our knowledge of the neurochemical environment along with cognition and functional connectivity across age in the posterior cingulate cortex (PCC). We therefore have chosen to use proton magnetic resonance spectroscopy (^{1}H-MRS) and functional magnetic resonance imaging (fMRI) together with a neuropsychological assessment battery to investigate the ageing process. The first part of the introduction will offer a review of the ageing literature in regard to ^{1}H-MRS and fMRI. This will be followed by the thesis aim, research questions and methodology. The last part of the introduction will cover the outline of the subsequent, self-contained chapters 2, 3, 4, 5, and 6, followed by the dissemination of research findings.

1.2. Ageing

The first part of the introduction provides a review of the literature addressing age-related changes in neurochemistry along with metabolite acquisition as well as functional connectivity, and how these have been linked to cognition.

It is well documented that the human brain undergoes changes over the course of its lifespan (Raz et al., 2005; Resnick et al., 2000; Salat et al., 2004). Older adults experience a decline in their cognitive functions, specifically memory, when compared to younger adults (Drag & Bieliauskas, 2010). Research has reported that some individuals with mild cognitive impairment (MCI), a pre dementia stage, may go on to develop Alzheimer's disease whereas others will remain stable or revert back and experience no memory problems (Gauthier et al., 2006). It is therefore imperative to understand the ageing process as normal and pathological processes might overlap. Advances in neuroimaging techniques have allowed the measurement and monitoring of age-related changes in regard to structure, neurochemistry, and functional connectivity. Structurally, magnetic resonance imaging (MRI) studies demonstrated global thinning of the ageing brain as well as area specific atrophy, such as in the regions of caudate, cerebellum, hippocampus, and prefrontal regions (Giorgio et al., 2010; Raz et al., 2005; Salat et al., 2004). Research has reported that global thinning is not associated with neuronal loss and suggested a possible breakdown of the neuronal and dendritic architecture (Freeman et al., 2008).

1.2.1. Ageing and transverse relaxation

The human brain uses axons to carry neurochemical information from one neuron to another to execute daily cognitive functions (Huettel, Song, & McCarthy, 2009). The neurochemical profile includes metabolites and neurotransmitters that support the neuronal processes across the brain (Duarte, Lei, Mlynárik, & Gruetter, 2012). To acquire data regarding these metabolites, ^{1}H-MRS takes advantage of the magnetic properties of hydrogen (^{1}H) atoms to measure metabolites in the brain region of interest (Hore, 2015). The most commonly reported metabolites include *N*-Acetylaspartate (NAA), choline (Cho, which consists of choline, glycerophosphocholine, and phosphocholine), and creatine (Cr, which is a combination of creatine and phosphocreatine) along with the neurotransmitters glutamate (Glu) and γ-Aminobutyric acid (GABA) (Rae, 2014). Metabolites and neurotransmitters are located in neuronal cell bodies and axons, and are involved in intra- and extracellular processes. Hence, alterations in metabolite concentrations might suggest changes at a cellular level. Moreover, transverse relaxation (T_2) time, which is an essential factor in metabolite quantification, might also provide information of the cellular microenvironment due to its sensitivity to alterations in molecular passage (Öngür et al., 2010). T_2 relaxation represents the time it takes for a signal to decay towards its equilibrium. The process occurs after a 90° radiofrequency (RF) pulse is applied to ^{1}H spins flipping them into the x-y plane (Hore, 2015). Here, the ^{1}H spins gain transverse magnetization and subsequently lose it resulting in signal loss. A more comprehensive explanation is provided in Chapter 3. As T_2 relaxation influences signal it is crucial to account for it when quantifying metabolites. Errors can be introduced if T_2 is not appropriately corrected and may result in confounding findings across age- or disease-related metabolite concentration differences (Barker et al., 1993; Rutgers & Van der Grond, 2002). T_2 literature has reported several T_2 relaxation values for singlet metabolites NAA, Cr, and Cho as well as scalar coupled metabolites such as Glu, GABA, and MI. However, it is not apparent from the reported T_2 relaxation values, which are definitive or representative of brain regions containing mostly white matter (WM), mostly grey matter (GM), or both. In addition, previous research investigating age effects of metabolite T_2 relaxation times has resulted in mixed findings. For example, it has been reported that NAA, Cr, and Cho T_2 relaxation times are either not changed, decreased, or increased with age (Brooks et al., 2001; Christiansen, Toft, Larsson, Stubgaard, & Henriksen, 1993; Kirov, Fleysher, Fleysher, Patil, Liu, & Gonen, 2008; Kreis, Slotboom, Hofmann, & Boesch, 2005; Longo, Bampo, Vidimari, Magnaldi, & Giorgini, 1995; Marjańska, Emir, Deelchand, & Terpstra, 2013). Presently, T_2 relaxation times have not been reported for Glu across age.

Therefore, an overview of the existing T_2 relaxation research is valuable for accurate metabolite quantification as is the investigation of Glu T_2 relaxation times with age.

1.2.2. Ageing and metabolite concentrations

At the early stages metabolite concentrations were measured in vitro from animal brain tissue extracts. With the advancement of ^{1}H-MRS, metabolite concentration levels were acquired in vivo from animal and human brains (Öz, Tkáč, & Uğurbil, 2013). Rodent studies have shown age-related increases in hippocampus Cho concentration levels as well as observed differences in Cho transport (Katz-Brull, Koudinov, & Degani, 2002). A further rodent study reported not only of age but also gender differences of mI and lactate concentrations in the cerebellar cortex and striatum (Zhang, Wu, Liu, & Zhang, 2013). In addition, increased Glu dehydrogenase 1 concentration levels have been observed in striatum and hippocampus (Choi et al., 2014). It is important to be aware of regional as well as metabolite concentrations levels differences when applying animal data to humans. Animal studies are useful in identifying disease biomarkers as well as help with development of drug treatment in preclinical studies (Öz et al., 2013). An important factor to consider is the design of animal models, as they have to be reproducible to human disease pathology and phenotype.

In humans, age-related metabolite changes have been investigated across the brain at varying magnetic field strengths. A three-year longitudinal study reported significant increases in frontal WM myo-Inositol (MI) concentrations in elderly males but no changes in NAA, Cr or Cho concentrations in frontal WM or occipitolparietal GM either across gender, or after a three-year period (Ross, Sachdev, Wen, & Brodaty, 2006). One of the limitations of this study included lack of tissue segmentation even though the two chosen voxels were not purely WM or GM. More recent research has suggested no age-related NAA concentration changes across the whole brain (Wu et al., 2012), PCC and hippocampus (Reyngoudt et al., 2012), whereas another study reported a positive correlation between PCC's NAA and age (Chiu et al., 2014). Moreover, Cr concentrations have been reported to increase with age while Cho concentrations are suggested to decrease or increase with age (Chiu et al., 2014; Reyngoudt et al., 2012). Potential differences between the studies that might have impacted on the results included methodological and technical differences such as processing and fitting parameters. A systematic review by Haga et al. (2009) revealed that majority of the studies investigating frontal brain region reported no significant metabolite alterations in older individuals. However, when conducting a meta-analysis on four out of the 18 studies included

in the systematic review, it suggested a trend in frontal NAA decline while a significant increase was observed in parietal Cr and Cho. The authors highlighted that only seven out of 18 studies performed tissue segmentation of WM and GM as well as cerebrospinal fluid (CSF). The majority of these studies performed data acquisition with a 1.5 Tesla (T) scanner, while only two studies used a 2T scanner. The signal to noise ratio (SNR) increases with increased magnetic field strength allowing the detection of smaller metabolite signals and better chemical shift dispersion, however this is somewhat counteracted by increased linewidths of the resonances (Barker, Hearshen, & Boska, 2001; Li et al., 2013). This is an important factor, as peak height of metabolites is inversely proportional to linewidth (Baker et al., 2001).

Age related alterations have also been observed in Glu, GABA, and glutamine (Gln) concentrations, although less research has focused on these neurotransmitters (Aufhaus et al., 2013; Chang, Jiang, & Ernst, 2009; Gao et al., 2013; Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013; Kaiser, Schuff, Cashdollar, & Weiner, 2005). Researchers have reported a decline in Glu concentrations in the motor cortex (Kaiser et al., 2005), parietal GM, basal ganglia (Chang et al., 2009), striatal (Zahr et al., 2013), anterior cingulate cortex (ACC), and hippocampus (Hädel et al., 2013). A trend increase was observed in Gln within the corona radiata, while Gln in the ACC correlated positively with age (Hädel et al., 2013; Kaiser et al., 2005). Investigations into GABA concentration have revealed that GABA+ (plus macromolecules) decreases in frontal and parietal regions (Gao et al., 2013), however only a trend was observed in the ACC (Aufhaus et al., 2013). The authors indicated that the trend for an increase in GABA+ was mainly due to macromolecules rather then GABA itself (Aufhaus et al., 2013).

We observed that a primary limitation for a number of studies was missing tissue segmentation (Haga, Khor, Farrall, & Wardlaw, 2009; Ross, Sachdev, Wen, & Brodaty, 2006). A substantial and growing literature suggests varying findings for metabolite T_2 relaxation times across brain tissue (see Chapter 3). As previously mentioned, T_2 relaxation times are necessary for metabolite quantification and the lack of consensus values may introduce errors in determining metabolite concentrations. It is therefore vital to establish consensus metabolite T_2 values in regard to tissue content and potential change with age to acquire accurate metabolite concentrations.

1.2.3. Cognitive ageing with neuroimaging focus

Research into cognitive ageing has predominantly focused on memory due to increased longevity and associated diseases such as dementia (Park & Festini, 2010). Most of these studies examined older adults performance on memory related tasks while comparing them to younger adults. Notably, research on cognitive performance, measured outside of the scanner, has reported older adults performing poorly on verbal and non-verbal episodic memory tests that require retrieval of previously experienced events (Tulving, 1984). Difficulties on memory tests may arise due to problems occurring at one of the episodic memory stages: encoding (storage of information acquired through visual, acoustic, and semantic stimuli), retention (maintenance of acquired memory), and retrieval (remembering stored information from memory) (Lezak et al., 2012; Naveh-Benjamin & Kilb, 2014; Tulving & Craik, 2000). Memory can be separated into two categories: short-term and long-term memory (Atkinson & Shiffrin, 1971; Cowan, 2008). Short-term memory is used to hold a small amount of information for a temporary time. Conversely, long-term memory stores a wealth of information from prior events and experiences. Within long-term memory, one can distinguish between declarative (explicit) and nondeclarative (implicit) memory. Declarative memory accesses conscious recollections of events and facts, whereas, nondeclarative memory is expressed as "how to" knowledge or skill memory (e.g. tying a shoe or driving a car) (Lezak et al., 2012, pp. 32). Within the framework of declarative memory, one can distinguish between semantic (e.g. practical knowledge) and episodic (e.g. autobiographical) memory (Cowan, 2008; Squire & Zola, 1996). Cognitive task measures have been developed to assess a person's memory to examine cognitive deficits (Nyberg, Bäckman, Erngrund, Olofsson, & Nilsson, 1996; Wechsler, 1945). Several studies, which have used the Wechsler Memory Scale (WMS; Wechsler, 1945), have reported of a drop in recall of immediate and delayed verbal and spatial tasks (Haaland, Linn, Hunt, & Goodwin, 1983; Haaland, Price, & Larue, 2003). Furthermore, age-related decline was observed in sensory acuity, processing speed, and spatial abilities (Naveh-Benjamin & Kilb, 2014; Pak, Czaja, Sharit, Rogers, & Fisk, 2008; Salthouse, 2000). While episodic memory has been observed to change with age, semantic memory has been reported to stay stable or improve with age (Nyberg, Bäckman, Erngrund, Olofsson, & Nilsson, 1996). Both episodic and semantic memory tasks have been linked to medial temporal lobe and prefrontal contribution activation in patient studies and animal work (Squire, Stark, & Clark, 2004). All in all, age effects have been observed in the following domains: working memory, long-term memory, processing speed, and inhibitory control (Reuter-Lorenz & Park, 2010).

The implementation of functional neuroimaging has allowed the creation of new theories of cognitive ageing. Li et al. (2001) reviewed previous literature to propose a theoretical link between neuromodulation, cognition, and behaviour. The authors' theory focuses on decreased dopaminergic modulation, which results in increased neuronal noise in transmission and less apparent neural representation with age.

Thereafter, Cabeza (2002) has proposed the hemispheric asymmetry reduction in older adults (HAROLD) model, which posits that older adults experience decreased neurofunctional lateralisation during cognitive performance in the prefrontal cortex (PFC) compared to younger adults. The evidence for the model has been derived from functional neuroimaging and behavioural studies investigating the domains of working memory, inhibitory control, episodic memory retrieval, and perception. The author further proposed that an age-related reduction in hemispheric lateralisation may possibly be explained through compensatory or dedifferentiation processes. Based on the evidence provided the HARALD model is limited to the PFC, however, the author suggests that the model could be extended to other brain areas (Cabeza, 2002).

In contrast, the compensation-related utilization of neural circuits hypothesis (CRUNCH) model, proposed by Reuter-Lorenz and Cappell in 2008, suggests that an ageing brain requires an increase in neuronal resources to accomplish the same task as a younger brain. This mainly applies to low-level task demands resulting in overactivation. However, increased demands result in a ceiling effect for older adults, followed by a drop in performance levels and underactivation compared to younger brains. The model suggests possible age-related compensatory processes (Berlingeri, Danelli, Bottini, Sberna, & Paulesu, 2012; Reuter-Lorenz &Cappell, 2008).

Park and Reuter-Lorenz (2009) proposed the Scaffolding Theory of Aging and Cognition (STAC), which assumes that the ageing brain builds compensatory scaffolding in events of cognitive, neural, and functional challenges over the lifespan. These challenges enclose white matter deterioration, atrophy, dopamine receptor depletion as well as dedifferentiation and default network dysregulation. In this context, compensatory scaffolding refers to building new or alternative neural circuits. The original STAC model has been revised to incorporate life-course factors and is referred to as STAC-r (Park & Reuter-Lorenz, 2014). Life-course factors are experiences gathered during an individual's life span, which may have an impact on the structure and function of the ageing brain as well as compensatory scaffolding.

Braver, Paxton, Locke, and Barch (2009) proposed the dual mechanism of control (DMC) theory, which focuses on proactive versus reactive modes of white matter and cognitive control in the PFC. Proactive control occurs prior to a cognitive event considered early selection, while reactive mode is employed after a cognitive event has occurred on a as needed basis considered as late correction (Braver et al., 2009; Jacoby, Kelley, & McElree, 1999). Braver et al. (2009) reported that older adults experienced altered PFC function compared to younger adults, which suggests that reactive control is used by a far greater extend in older than younger adults.

Research has also investigated cognitive performance and neurometabolite changes across age. Ferguson et al. (2002) reported that performance on memory tests, such as Logical Memory, delayed 24h Logical Memory and Verbal Memory Factor, was positively correlated with higher NAA/Cr and Cho/Cr concentration ratios in healthy elderly men. Furthermore, performance on memory tests, such as Visual Reproduction, Visual Retention Test, and Auditory-Verbal Memory Test, has been positively linked with Cho/Cr ratio. Zahr et al. (2008) reported that poor performance on fluency and working memory was positively correlated with lower striatal Glu levels, whereas reduced striatal Glu concentration was negatively correlated with performance on set shifting. The authors also reported that striatal Glu concentration is a predictor of performance on the Grooved Pegboard (Zahr et al., 2013). There is limited in vivo research investigating both Glu and GABA concentration levels with cognitive performance. Therefore, it remains to be determined how the major excitatory and inhibitory neurotransmitters may play a role in cognitive performance in ageing.

1.2.4. Metabolites and BOLD in ageing

Blood-oxygen-level dependent (BOLD) contrast is commonly used in fMRI studies by taking advantage of blood susceptibility changes to measure task-based or resting-state activity (Bandettini, 2012; De La Iglesia-Vaya, Kanaan, Molina-Mateo, Martí-Bonmati, & Escarti-Fabra, 2013). The acquisition of neuronal activity is much slower compared to actual cognitive functions, however fMRI has provided major contributions to the understanding of the ageing process (Park & Reuter-Lorenz, 2009). Task-based fMRI studies provide information of neuronal activity changes while participants engage in tasks that test their cognitive, emotional or motor skills (Huettel, Song, & McCarthy, 2009).

As previously mentioned, GABA is a neuromodulator and acts to prevent excitatory activity, however GABAergic interneurons are only represented by 15-20% while the reminder are glutamatergic cortical neurons (Buzsáki, Kaila, & Raichle, 2007). Research has suggested that the balance between excitatory and inhibitory neurotransmitters is indirectly linked to the BOLD signal (Buzsáki, Kaila, & Raichle, 2007). Previous research has examined the relation of Glu and GABA to BOLD signal activity in task-based and resting-state fMRI. Resting-state GABA concentration has been shown to positively correlate with task-based negative BOLD signal in the ACC (Northoff et al., 2007). Further studies reported of an inverse relation between task-based BOLD signal and resting-state GABA in the medial occipital cortex (Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009), visual cortex (Stagg, Bachtiar, & Johansen-Berg, 2011), and primary motor cortex (Donahue, Near, Blicher, & Jezzard, 2010). In addition to reports of negative correlation between resting-state GABA concentrations and task-based BOLD signal it has been reported that haemodynamic response functions width correlates positively with resting-state GABA concentrations suggesting a representation of differences in neuronal activity (Muthukumaraswamy, Evans, Edden, Wise, & Singh, 2012). Arrubla et al. (2014) reported a negative correlation between resting-state GABA/ Cr+PCr ratio in the PCC and the connectivity strength of putamen to the default mode network (DMN). Harris et al. (2015) examined GABA concentrations in relation to task-based BOLD signal in five brain regions including occipital cortex, auditory cortex, sensorimotor cortex, frontal eye field, and dorsolateral prefrontal cortex. Their findings suggest no relation of GABA concentrations with BOLD signal in the five regions. Research combining Glu and GABA measures in the posteromedial cortex reported of Glu/Cr ratio correlating positively and GABA/Cr ratio correlating negatively with DMN intrinsic functional connectivity (Kapogiannis, Reiter, Willette, & Mattson, 2013). These studies examined Glx, Glu, and GABA concentrations in relation to task-based and resting-state BOLD signal in single groups, which were primarily represented by younger adults. Therefore, it appears unclear if the relations between Glu and GABA with BOLD signal are age dependent.

1.2.5. BOLD and cognition in ageing

A substantial and growing ageing literature reports a decline in the framework of memory functions, specifically in working and episodic memory, in older compared to younger adults (Cabeza, et al., 2004). fMRI research, utilizing memory tasks, observe increased brain activity in ACC and parietal cortex in older compared to younger adults, while reduced activity was observed in hippocampus and occipital cortex (Cabeza, et al., 2004; Daselaar, Fleck, Dobbins, Madden, & Cabeza, 2006; Sharp, Scott, Mehta, & Wise, 2006). Research

investigated prefrontal activity utilizing recall and source memory tasks in younger adults as well as older adults whose performance was either low or high on the memory tasks (Cabeza, Anderson, Locantore, & McIntosh, 2002). The authors reported that younger and low performing older adults on source memory had activity in the right prefrontal cortex, while high performing older adults engaged the left and right prefrontal cortex. The authors further suggested that bilateral region use might imply that older adults compensate for cognitive decline.

Reports from resting-state fMRI studies have demonstrated activity in a network of brain regions in comparison to the rest of the brain when a participant is given no other instructions than to think to themselves (Buckner, Andrews- Hanna, & Schacter, 2008). These brain regions comprise the DMN and include ventral medial prefrontal cortex, posterior cingulate/ retrosplenial cortex, inferior parietal lobule, lateral temporal cortex, dorsal medial prefrontal cortex, and hippocampal formation. The DMN has been linked to memory encoding and retrieval through overlap of brain regions in the medial temporal lobe (Squire, Stark, & Clark, 2004). Strong connectivity links have been found between the PCC and medial temporal lobe as well as the retrosplenial cortex (Fransson & Marrelec, 2008; Greicius, Supekar, Menon, & Dougherty, 2009). The PCC plays a central role in DMN with high activity at rest (Raichle et al., 2001). Interestingly, research has implicated the PCC with episodic memory processing and autobiographical memory retrieval despite it previously not being thought of as being involved in declarative memory (Dunn et al., 2014; Maddock, Garrett, & Buonocore, 2001). The PCC is part of the cingulate gyrus, which is superior to the dorsal corpus callosum (Leech & Sharp, 2014). The PCC, precuneus, and retrosplenial cortex form the posteromedial cortex. On the basis of cytoarchitectonics the PCC is associated with Brodmann areas 23 and 31, and considered a paralimbic cortical structure (Brodmann, 1909). Previous non-human research has established efferent cortical projections between medial temporal lobe (MTL) and cingulate/ retrosplenial cortices (RSC) in macaque monkeys (Lavenex, Suzuki, & Amaral, 2002). A study by Greicius et al. (2009) combined fMRI and diffusion tensor imaging (DTI) to investigate structural and functional connectivity in the default mode network in humans. The authors suggested that fibers from MTL enter the PCC/ retrosplenial cortex (RSC) as previously supported by animal studies. Here, the regions of interest in the MTL included the hippocampus, parahippocampal and entorhinal cortex.

Dunn et al. (2014) reported that individuals with amnestic mild cognitive impairment who had difficulties with episodic memory retrieval also had a lack of connectivity between the

hippocampus and PCC. The authors suggested that hippocampal atrophy is not uniquely responsible for DMN impairment but in combination with the PCC. Damoiseaux et al. (2008) investigated the DMN in younger and older adults at rest. The authors reported of reduced DMN activity along with decreased grey matter in older compared to younger adults. It is therefore important to investigate the PCC and hippocampus, as they seem to have integral involvement in memory and old age.

1.3. Thesis aim, research questions, and methodology

The aim of the thesis is to investigate the ageing process through the neurochemical changes (T_2 relaxation times and concentrations) as well as the link between neurochemical environment and functional connectivity in the brain. The purpose of this thesis is to reduce gaps in knowledge but at the same time provide ideas for future research in regard to the ageing population along with neuroimaging techniques.

This thesis sets out to answer the following research questions:

1.) What are the consensus neurometabolite T_2 values across brain tissue content?
2.) Do neurometabolite T_2 values differ between younger and older adults and how might this impact metabolite quantification with ^{1}H-MRS?
3.) Do excitatory and inhibitory neurotransmitters predict cognitive performance across age?
4.) What is the relationship between excitatory and inhibitory neurotransmitters and functional connectivity across age?

To address research question 1, data was combined from studies, which examined metabolite transverse relaxation in the human brain using ^{1}H-MRS.

Research question 2 is assessed with new collected data from healthy younger and typically ageing older adults. This study investigates neurometabolite T_2 relaxation values between younger and older cohorts.

To assess research question 3, the findings from research questions 1 and 2 were incorporated in methods, specifically, in metabolite quantification. Data from a new and larger

cohort was used to address research question 3. Neuropsychological assessment and ^{1}H-MRS data were collected to assess any relation between both measures with age.

Research question 4 builds on the findings from research question 3 by including functional neuroimaging data. The same cohort as in research question 3 was used. Here, the question was addressed by determining any relation between neurochemical (^{1}H-MRS) and functional (fMRI) data across age.

1.4. Outline of the thesis

The framework of the thesis contains the following six chapters: a general introduction, general methods, a systematic review, three empirical chapters, and a final discussion of the findings from preceding chapters. Furthermore, chapters 3 to 6 have been adapted in a format of journal articles, as chapter 4 has been submitted to a peer-reviewed journal, and chapters 3, 5, and 6 will be prepared for publication after thesis submission. The material presented in this thesis covers the same research area; therefore some duplication of information will be present. The summary of each chapter is as follows:

Chapter 2 – General methods

Chapter 2 offers supplementary background information to neuroimaging concepts as well as providing details of ethical approval, participant recruitment, and description of neuropsychological assessment battery.

Chapter 3 - A systematic review of ^1H metabolite T_2 relaxation time in healthy human brain

Chapter 3 presents the results of a systematic review of studies investigating metabolite T_2 relaxation times with the utilization of ^{1}H-MRS in the human brain. This review provides an overview of proton metabolite T_2 values in brain tissue, as well as addressing possible difficulties regarding T_2 research and proposing consensus T_2 values for neurometabolites (NAA, Cr, Cho, mI and Glu) in grey matter, white matter, and mixed tissue content.

Chapter 4 - Posterior Cingulate Gyrus T_2 Relaxation Times and Concentration Levels of Proton Metabolites in Ageing Brain at 3 Tesla

Chapter 4 examines possible age-related effects on measures of metabolite T_2 relaxation times within the PCC and associated effects this has on concentration estimation. ^{1}H-MRS was utilized to acquire NAA, Cho, Cr, Glu, and mIn T_2 values and concentration measures from healthy young and typically ageing older adults. The outcome will be referred to by $T_2^\dagger$, as external and internal factors affect apparent decay. The results suggest NAA $T_2^\dagger$ relaxation exhibits age-related decline, suggesting micro-environmental changes within PCC's neurons. When using age appropriate $T_2^\dagger$ values for relaxation correction in metabolite concentration estimation, previous age-related declines in NAA was no longer seen. These findings re-enforce the importance of T_2 relaxation measurements and the use of appropriate relaxation corrections in MRS quantification.

Chapter 5 - Glutamate and GABA Levels in Relation to Cognitive Performance Across Age

Chapter 5 examines the concentrations for the major excitatory (Glu) and inhibitory (GABA) neurotransmitters in relation to cognitive performance. Proton magnetic resonance spectroscopy was used to acquire Glu and GABA concentrations from the PCC after the healthy younger and older adults completed cognitive assessments. The results showed older adults performing significantly worse on immediate and delayed visual reproduction as well as the Stroop interference task compared to younger cohort. Reduced Glu concentration levels in the PCC were predictive of performance on immediate and delayed visual reproduction. However, there was no correlation between GABA+ (plus macromolecules) concentration and cognitive performance across age. The results indicate that a decline in Glu concentration levels may indirectly be linked with cognitive processes.

Chapter 6 – Resting-state glutamate and GABA in relation to functional connectivity interrelations across age

Chapter 6 investigates the association of Glu and GABA+ concentrations with functional connectivity between the PCC and hippocampus across age. The same cohort was used as in Chapter 5 with an additional fMRI scan. The result suggests a positive association between resting-state PCC Glu concentrations and functional connectivity between PCC and hippocampus across age, while there was no significant correlation with GABA+ concentrations. This outcome broadly supports the theory of glutamatergic involvement in functional connectivity in the human brain.

Chapter 7 – General Discussion

Chapter 7 is a summary and discussion of the key findings from the systematic review and empirical studies as well as practical implications, limitations, and recommendations for future direction.

1.5. Dissemination of research findings

Chapter 4 has been submitted for publication in a peer-reviewed journal.

> K.W. Rusiak, A.L.W. Bokde, J.P. McNulty, and P.G. Mullins (2016). *Posterior Cingulate Gyrus T_2 Relaxation Times and Concentration Levels of Proton Metabolites in Ageing Brain at 3 Tesla.*

Two abstracts have been published in *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, which are based on the findings from the data included in this thesis.

> Rusiak, K., Mullins, P. G., Bokde, A. L., & McNulty, J. (2015). Transverse relaxation times of proton metabolites in ageing brain at 3 Tesla: Effects on previously reported metabolite changes. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, *11*(7), P100. http://dx.doi.org/10.1016/j.jalz.2015.06.170
>
> Rusiak, K., Kehoe, E. G., McNulty, J., Bokde, A. L., & Mullins, P. G. (2014). Posterior Cingulate Glutamate Changes in Aging: A 1H-MRS Study. *Alzheimer's & Dementia: The Journal of the Alzheimer's Association*, *10*(4), P114. http://dx.doi.org/10.1016/j.jalz.2014.05.218

Further findings from the data contained in this thesis have been presented at several conferences and symposiums:

> Rusiak, K., Bokde, A.L., McNulty, J., Mullins, P.G., (2015, July). *Posterior cingulate glutamate changes in aging: a ^{1}H-MRS study*. Poster session presented at the Alzheimer's Association International Conference 2015, Washington DC, USA.
>
> Rusiak, K. (2015, June). *Chaining the aged brain: Structural restrictions evidenced through chemical and functional alterations across age*. Thesis presented at the Year Three PhD Student Conference by School of Psychology, Bangor University, Bangor, UK.
>
> Rusiak, K., Kehoe, E.G., McNulty, J., Bokde, A.L., Mullins, P.G., (2014, July). *Posterior cingulate glutamate changes in aging: a ^{1}H-MRS study*. Poster session

presented at the Alzheimer's Association International Conference 2014, Copenhagen, Denmark.

Rusiak, K., Bokde, A.L., McNulty, J., Mullins, P.G., (2014, April). *Metabolic changes in the posterior cingulate gyrus across age: a ^{1}H-MRS study.* Pitch/Poster session presented at the 23rd British Chapter ISMRM Postgraduate Symposium, Cardiff, UK.

1.6. Conclusions

Ageing is accompanied by cognitive and neurophysiological changes, which can impact on an individual's life. Advancement in neuroimaging has reported of alterations in neurochemical concentration levels and neuronal activity with age. To gain insight into the ageing mechanisms the thesis will explore neurochemical changes across age along with cognitive performance and functional connectivity.

Firstly, to estimate accurate metabolite concentration levels, T_2 relaxation time has to be taken into consideration. Despite the existence of substantial literature on T_2 relaxation, there are no consensus metabolite T_2 values. Therefore, there is a need to investigate metabolite consensus T_2 relaxation values across tissue content. Also, it is unclear if metabolite T_2 relaxation values alter across age.

Furthermore, research suggests concentration alterations of commonly investigated metabolites such as NAA, Cr, and Cho. However, there is limited research on the major excitatory and inhibitory neurotransmitters, Glu and GABA, respectively. Therefore, it remains to be determined how the major excitatory and inhibitory neurotransmitters may play a role in cognitive performance in ageing.

Finally, it is unclear if Glu and GABA alterations may relate to the functional activity in areas active during cognitive performance. Therefore, another aim of the thesis is to investigate the link between Glu and GABA with functional connectivity across age.

The findings from ^{1}H-MRS and fMRI can improve our understanding and better characterise age-related changes while providing underpinnings of cognitive functions.

Chapter 2

General Methods

2.1. Introduction

This chapter provides supplementary background information of the methods used in the systematic review and empirical studies. The first part of the general methods will offer a brief description of the neuroimaging concepts used throughout this thesis, which include nuclear magnetic resonance (NMR), ^{1}H-MRS and fMRI. Subsequently, ethical approval and participant recruitment will be provided in detail, followed by a description of the neuropsychological assessment battery used to test cognitive performance.

2.2. The Principles of Nuclear Magnetic Resonance

This first part of the general methods section will briefly describe the basics of nuclear magnetic resonance followed by ^{1}H-MRS and fMRI acquisition (following in 2.2.1 and 2.2.2).

Felix Bloch (1946) and Edward Purcell (1946) independently discovered nuclear magnetic resonance (NMR) in 1946, for which both were awarded the Nobel Prize in Physics six years later (Huettel, Song, & McCarthy, 2009). To understand the principle of NMR the composition of the atom has to be considered. Each atomic nucleus consists of a different number of protons and neutrons while the electrons orbit around the core and are negatively charged (Bloch, 1946; Hore, 2015; Purcell, 1946; Roberts, 1959). These subatomic particles posses a spin, or angular momentum, which creates an electromagnetic field with either a positive or negative charge. Some atomic nuclei have no spin due to opposite signs (+/-) pairing and cancelling out, while other nuclei will have either a half-integer spin (e.g. 1/2, 3/2) or integer spin (e.g. 1, 2), this is dependent on the number of unpaired protons and neutrons in the nucleus. Sometimes referred to collectively as "spins", these nuclei have a random orientation, however, when placed in an external main magnetic field (B_0) will align with (spin up) or against (spin down) the magnetic field and posses a magnetic moment. Nuclei in the 'spin up' state are at a lower energy state then 'spin down' nuclei, and so more spins align with the field, (spin up), producing an overall net magnetization aligned with the main magnetic field. The energy difference between the two spin states will increase with higher field strength, and so at higher field there will be more spins in the lower energy state, producing a greater overall net magnetization aligned with the field. Even though the spins align with the external magnetic field, they still have a spin (angular momentum) as they precess around the main magnetic field (Figure 1).
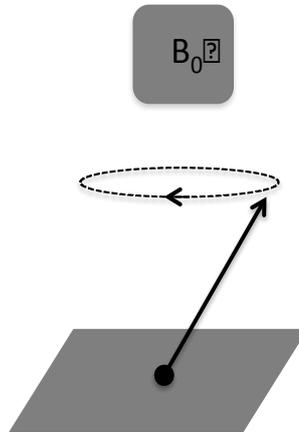
Figure 1. Illustration of a spin precessing around the B_0.

The frequency of precession (ω, unit: MHz) is determined by the gyromagnetic ratio (γ, unit: MHz/Tesla) and the strength of the external magnetic field (unit: Tesla) the spins are exposed to. This can be expressed as the Larmor frequency equation:

$$\omega = \gamma \, B_0$$

The precession process can be demonstrated by the rotating frame of reference using the Cartesian co-ordinate system (x, y, z) (Bloch, 1946; Purcell, 1946; Roberts, 1959). The B_0 is depicted as the z-axis, from a stationary point of view, while the xy-plane moves around the z-axis (Figure 2.a). As the spins align with the B_0 they experience net magnetization (M) or state of equilibrium in the z-plane until a second oscillating magnetic field (B_1) (e.g. radio frequency pulse) is introduced. Now, the spins in lower state absorb the energy from B_1 and flip to a higher energy state. However, this is only possible if B_1 is in the same resonance frequency as the nucleus. As the spins absorb the energy they are flipped into the transverse plane (xy-plane) and experience transverse magnetization while subsequently rotating with and around the xy-plane (Figure 2.b).

19
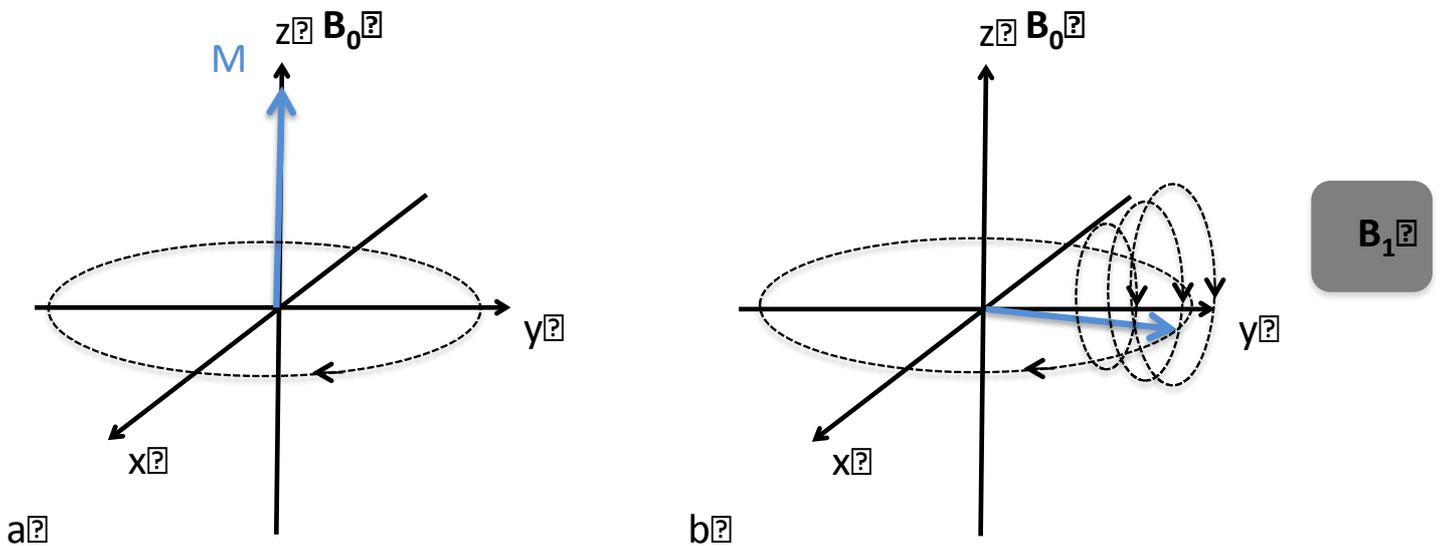
Figure 2. (a) Spins experience net magnetization (M) in the B_0 from a stationary point of view, while the xy-plane rotates around the z-axis. (b) After the application of the B_1 the spins rotate around and with the xy-plane.

When B_1 is removed or switched off, the spins will relax and return to its lower state (equilibrium) while emitting excess energy that can be measured (Bloch, 1946; Hore, 2015; Purcell, 1946; Roberts, 1959). Two important processes prompt decay in transverse magnetization. One of them is spin-lattice relaxation (also known as T_1 or longitudinal relaxation), whereas the other is spin-spin relaxation (referred to as T_2 or transverse relaxation). Both processes are commonly measured in milliseconds (ms) and represent the time it takes for all the spins to return to natural alignment. The process of absorbing and re-emitting energy is called nuclear magnetic resonance.

The different nuclei with a spin include hydrogen (^{1}H), carbon (^{13}C), phosphorus (^{31}P), sodium (^{23}NA), fluorine (^{19}F), chlorine (^{35}Cl) and potassium (^{39}K) (Hore, 2015.). However, the principal nucleus used in neuroimaging is ^{1}H as it provides high sensitivity due to its high natural abundance and magnetic sensitivity as well as possessing a high natural occurrence in the human body (Currie et. al, 2012; Soares and Law, 2009).

2.2.1. The Basics of Proton Magnetic Resonance Spectroscopy

Nuclear magnetic resonance spectroscopy (MRS) has been a useful tool in chemistry and physics before the development of magnetic resonance imaging (MRI) (Bovey, Mirau, & Gutowsky,1988; Mountford, Stanwell, Lin, Ramadan and Ross, 2010; Roberts, 1959). MRS is a useful tool not only in research but also clinical settings by characterising tumours or strokes as well as neurodegenerative diseases while monitoring the progression of some of these diseases. It is based on the same principle as NMR using the same nuclei, such as ^{1}H, ^{13}C, or ^{31}P, for acquisition. MRS is a non-invasive in vivo technique, which exploits the magnetic properties of nuclei of atoms and molecules to measure chemicals in the human body (Hore, 2015). Fortunately, no additional hardware is required, only the MRI system alongside additional software (Drost, Riddle, and Clarke, 2002). However, all the hardware and software have to be tuned to the radio frequency (RF) of the nucleus of interest. An MRI scan would provide an anatomical image of the brain while a ^{1}H-MRS scan will produce a spectrum of neurometabolites (Figure 3).
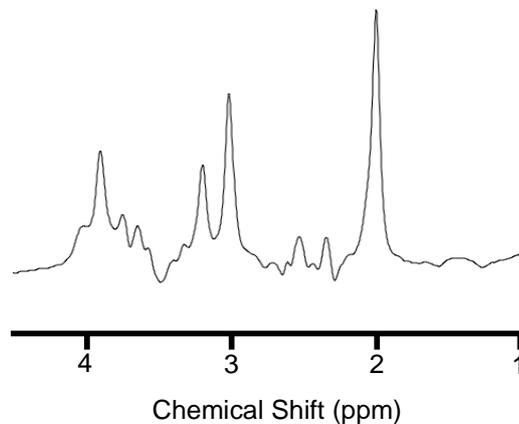
Figure 3. Averaged spectra acquired with a Point-RESolved Spectroscopy (PRESS) sequence utilizing ^{1}H-MRS.

It is valuable to try to understand that in spite of protons having the same magnetic moment they will not experience the same frequency (Bovey, Mirau, & Gutowsky,1988; Drost, Riddle, and Clarke, 2002; Hore, 2015). The frequency will be dependent on the chemical

environment or neighbouring atoms the protons are surrounded by. As noted in section 2.2., the frequency of nuclei can be determined with the Larmor equation and in this case would end up with only one peak on a NMR spectrum, for each type of nuclei detected. However, when electrons orbit the nucleus they create a secondary induced magnetic field, which opposes the B_0 resulting in a shielding effect. Therefore, nuclei experience different resonance frequencies from one molecule to another. To determine the frequency of precession (f) a screening constant σ_{cs} ($|\sigma_{cs}| \ll 1$) has to be taken into account. As a result, the Larmor equation is given by

$$f = \gamma \, B_0 \, (1 - \sigma_{cs})$$

The described variety of nuclei resonance frequencies forms the foundation of MRS (Bloch, 1946; Bovey, Mirau, & Gutowsky,1988; Drost, Riddle, and Clarke, 2002; Purcell, 1946;). In this context, the resonance for ^{1}H at 3 Tesla is 127.74 MHz (or 42.58 MHz/Tesla). The effect of protons experiencing different resonance frequency (shielding) due to their chemical environment is called chemical shift (δ). For standardisation purposes the unit of measure is reported in parts per million (ppm) and identifies the location of nuclei on the spectrum. Furthermore, a spectrum can also tell us more about the pattern of the chemical environment experienced by the nuclei (Govindaraju, Young, & Maudsley, 2000). The chemical environment of nuclei creates magnetic interactions between each nucleus due to their shared electrons, which is referred to as spin-spin coupling, J-coupling or scalar coupling (Bloch, 1946; Bovey, Mirau, & Gutowsky,1988; Hore, 2015). These interactions cause the splitting of the resonances and produce multiple peaks or multiplets. The ^{1}H-MRS signal is referred to as free induction decay (FID) and is acquired in the time domain. To create spectra the fast Fourier Transfer (FFT) algorithm is used to de-convolve FID's into spectral patterns in the frequency domain, which are displayed by the chemical shifts.

The acquisition of spectra can be accomplished either by single voxel (SVS) or multiple voxels, such as chemical shift imaging (CSI) or magnetic resonance spectroscopic imaging (MRSI) (Mandal, 2012). Commonly used ^{1}H-MRS acquisition techniques include Point-RESolved Spectroscopy (PRESS), Stimulated Echo Acquisition Mode (STEAM), and for quantification of GABA the edited technique MEscher-GArwood Point-RESolved Spectroscopy (MEGA-PRESS) (Bottomley, 1987; Frahm et al., 1989; Mescher et al., 1996, 1998). Both, PRESS and STEAM, make use of three slice selective RF pulses to determine the volume of interest. In the PRESS sequence the first pulse is 90° and is followed by two 180° pulses (Bottomley, 1987), whereas in the STEAM sequence all three pulses are 90°

(Frahm et al., 1989). In the STEAM sequence the time between the second and third RF pulse is referred to as mixing time (TM). The advantage of STEAM is the use of a shorter echo time (TE), while PRESS has the advantage of better signal-to-noise (SNR) ratio as the second pulse refocuses the spins in the transverse plane.

The metabolite GABA requires a special acquisition technique, as it has three coupled resonances at 1.9ppm, 2.28ppm, and 3.01ppm, which overlap with NAA/ NAAG, glutamate/ glutamine, and creatine, respectively. The MEGA-PRESS editing technique is the most commonly used method for detection of GABA (Mullins et al., 2014) and consists of two acquisitions, one 'ON' and one 'OFF'. During the 'ON' acquisition an additional editing-pulse is applied to the resonance peak at 1.9 ppm in a modified PRESS sequence. This makes use of the J-coupling of the peak at 1.9 ppm with the peak at 3.01ppm (Mescher et al., 1998; Mullins et al., 2014), leading to transfer of magnetisation and an increase in signal at the 3.01 peak. The OFF spectrum is collected with a similar additional RF pulse, in a symmetrical position around the water peak (at 7.5 ppm). By subtracting the 'ON' from the 'OFF' acquisition spectra most metabolite peaks cancel out, leaving an edited GABA peak, as well as a downward phased NAA peak, and combined peaks for glutamate and glutamine, denoted as Glx (Figure 4).

Of particular interest to ^{1}H-MRS is the presence of water in the brain (Kreis, 1997). The water signal concentration is about 3600 mM, whereas the signal for other metabolites is in the range of 1-10 mM (Drost, Riddle, & Clark, 2002). Given this, water suppression is required in order to avoid the water signal overpowering the metabolite signals. This is accomplished through applying a narrow band RF pulse (in the frequency of water) at the beginning of the desired acquisition technique (Drost, Riddle, & Clark, 2002; Kreis, 1997). A common water suppression technique used in ^{1}H-MRS is Chemical-Shift Selected (CHESS) pulses, which is followed by spoiler gradients to ensure no water signal will rise (Drost, Riddle, & Clark, 2002; Haase, Frahm, Hanicke, & Matthaei, 1985). Nevertheless, researchers have to be aware that water presaturation may affect metabolite signal (Kreis, 1997).
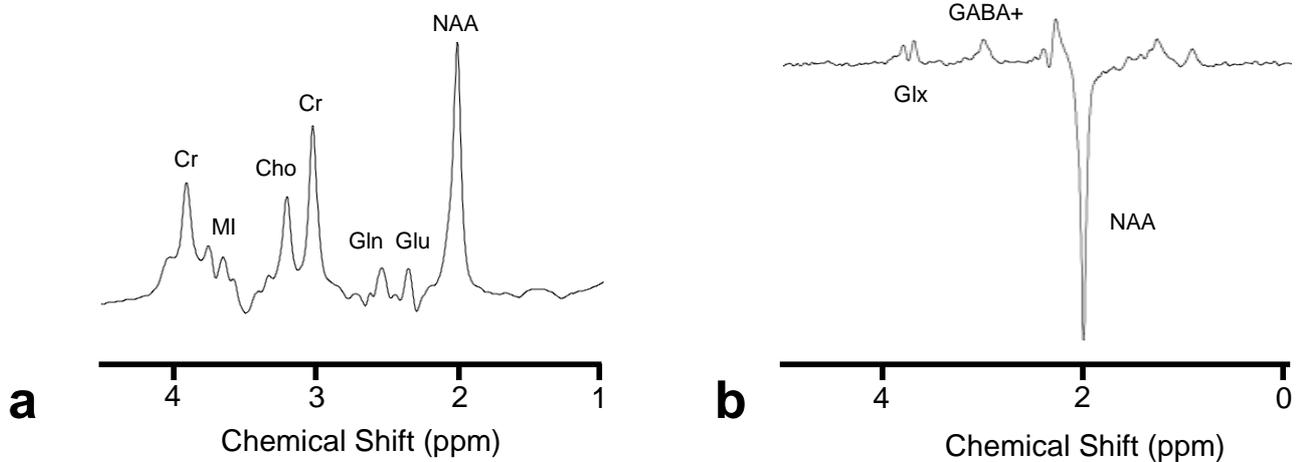
Figure 4. Sample spectra acquired with PRESS (a) and MEGA-PRESS (b) sequence utilizing ¹H-MRS.

The following metabolites are commonly reported in the literature and their principal characteristics will be considered.

N-acetylaspartate (NAA) is acetylated from the amino acid aspartate in neurons while it is de-acetylated primarily in oligodendrocytes and catabolised in glial cells (Mountford, Stanwell, Lin, Ramadan, & Ross, 2010; Rae, 2014). NAA has a high concentration compared to other free amino acids with a prominent peak resonating at 2.01 ppm while a further peak can be observed at 2.6 ppm. Additionally, it has small contributions from N-acetylaspartylglutamate (NAAG) resonant at 2.05 ppm. The role of NAA has been indicated as a neuronal marker due to its loss or decreases with disease (Gonen et al., 2000; Soares, & Law, 2009), however, there is still no clear definition of NAA's role.

Total choline (Cho) signal is composed of free choline, phosphocholine and glycerophosphocholine while resonating at 3.21 ppm (Mountford, Stanwell, Lin, Ramadan, & Ross, 2010; Rae, 2014). Choline is not synthesised in the brain and has to be supplied through diet and produced in the liver. It is considered to be a marker of membrane turnover due to changes observed in tumour pathology as well as during inflammations (Ross, &

Bluml, 2001). Research has suggested marginal differences in Cho concentrations in WM (1.6 mM) and GM (1.4 mM).

Creatine (Cr) has its primary resonance at 3.02 ppm (Mountford, Stanwell, Lin, Ramadan, & Ross, 2010; Rae, 2014). Its signal is a combination of creatine and phosphocreatine, whereby animal research has shown that it can be synthesised in the brain along with being taken up through diet (Braissant, Henry, Loup, Eilers, & Bachmann, 2001). Cr is commonly used as an internal standard as a ratio to other metabolites, however research suggests caution as it might change with age (Haga et al., 2009).

Glutamate (Glu) and **Glutamine (Gln)** create a complex of peaks called **Glx** (2.12 ppm – 2.35 ppm), due to the difficulty of separating both peaks at 1.5T (Ross & Bluml, 2001). As acquisition techniques have improved in conjunction with higher magnetic field strength it is possible to separate both metabolites while maintaining a good quality signal from both metabolites (Snyder & Wilman, 2010). Glu is the major excitatory neurotransmitter in CNS and predominantly found in neurons (Ross & Bluml, 2001). Glu is the precursor to Gln and GABA (Martinez-Hernandez, Bell, & Norenberg, 1977; Mountford, Stanwell, Lin, Ramadan, & Ross, 2010). An increased Glu accumulation can lead to excitotoxicity through which damage or cell death can occur (Besancon, Guo, Lok, Tymianski, & Lo, 2008).

γ-Aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the CNS and is synthesised through the action of glutamate decarboxylase (Mountford, Stanwell, Lin, Ramadan, & Ross, 2010; Rae, 2014). Sometimes GABA is also referred to in the literature as GABA+ due to the overlap with macromolecules (MM). In this thesis GABA will also be referred to as GABA+. As GABA+ overlaps with various metabolites such as NAA, Glu, Gln, and Cr a special editing sequence (MEGA-PRESS) has to be used for acquisition purposes (Mescher et al., 1996, 1998). GABA is a neuromodulator together with Glu, however GABAergic interneurons are only represented by 15-20% while the reminder are glutamatergic cortical neurons (Buzsáki, Kaila, & Raichle, 2007).

Myo-Inositol (mI) is a simple sugar and synthesised endogenously from glucose (Loewus & Loewus, 1983; Rae, 2014). It is resonating at 3.54ppm, while the concentration varies from brain region to brain region (Minati, Aquino, Bruzzone, & Erbetta, 2010). mI is suggested to be a glial marker, however, research differs on this topic as it can also be found in neuronal cells (Rae, 2014).

There are many more metabolites such as lactate, glycine, aspartate, and glutathione, which will not be covered in this thesis.

2.2.2. The Basics of Functional Magnetic Resonance Imaging

Functional magnetic resonance imaging (fMRI) is a widely used technique measuring physiological changes in correlation to neuronal activity (Huettel, Song, & McCarthy, 2009). The key element of almost all fMRI studies is the use of endogenous blood-oxygenation-level dependent (BOLD) contrast, which takes advantage of blood susceptibility changes (Bandettini, 2012).

Usually the participant will be either asked to perform a task or is exposed to stimuli, yet brain activity can also be measured at rest while the participant is lying still in the scanner. The information processing in the human brain is accomplished through neurons in the central nervous system (CNS) (Huettel, Song, & McCarthy, 2009). Their primary role involves integration by collecting information from the surrounding neurons via dendrites and cell body, and signalling back information to other neurons. Upon neuronal activity an increased supply of energy is required. This energy comes in the form of adenosine triphosphate (ATP), which in turn is synthesised from glucose and oxygen supplied through the vascular system via arteries, capillaries, and veins. Precisely, oxygen is bound to haemoglobin, a protein molecule, in red blood cells. Haemoglobin can have two states. The first is oxygenated haemoglobin that is bound to oxygen and hence has no magnetic moment, also referred to as diamagnetic. The second state is deoxygenated haemoglobin, which has no attached oxygen and therefore has a magnetic moment (paramagnetic). Oxygenated haemoglobin has no effect on its surrounding tissues and provides a higher signal. On the contrary, deoxygenated haemoglobin distorts the surrounding tissue due to its magnetic properties and results in a lower signal, which has previously been demonstrated by Thulborn, Waterton, Matthews and Radda in 1982. The distortion occurs due to a quick increase in apparent transverse relaxation (T_2^*) (please see Chapter 3 for additional T_2^* information). T_2^* weighted images are sensitive to oxygenated haemoglobin as they will darken with increased presence of deoxygenated haemoglobin. The BOLD signal changes in T_2^* weighted images can be convolved and represented by the hemodynamic response function (HRF) (Buxton, Uludağ, Dubowitz, & Liu, 2004). Figure 5 shows a typical shape of the HRF depicting a delayed response of oxygenated blood as a result of neuronal activity in the brain. When using BOLD contrast only T_2^* is important as longitudinal relaxation (T_1) is fully recovered.

As this part of the thesis has provided the basics of fMRI, readers are advised towards the paper 'Twenty years of functional MRI: The science and the stories' by Bandettini (2012), which provides a comprehensive overview of the history of fMRI and the different research groups that have helped to develop fMRI as a tool to understand how the brain works.
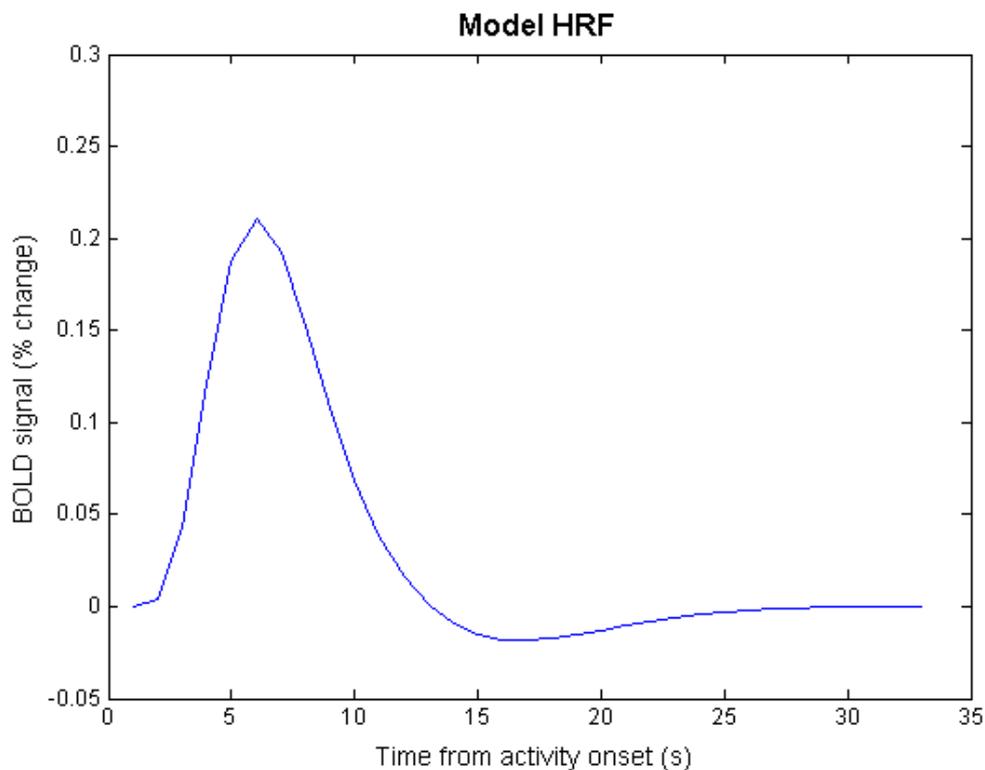
Figure 5. Simulated hemodynamic response curve demonstrating a short onset delay in signal followed after a few seconds by a rise to a peak resulting in a return to baseline with an undershoot. This process takes around 30 seconds and is dependent on the presented stimuli. It has to be considered that some researchers have reported the presence of an initial undershoot before the peak rise in BOLD signal.

2.3. Ethical approval and participant recruitment

The Bangor University School of Psychology Ethics and Research Committee granted ethical and governance approval for the study in Chapter 4 (2012-6522-A13630) and for the studies in Chapter 5 and 6 (2013-11044-A12103), which can be found in the Appendix A and B, respectively.

Potential participants of all ages were recruited from within Bangor University imaging unit, Bangor University School of Psychology participant panel, NEURODEM's participant register, by word of mouth from associates of the researchers, SONA (Appendix C), Facebook, flyers (Appendix D and E), local businesses (such as Morrisions, Blue Sky Cafe, etc.), and/ or local community (such as churches, community centre, etc.).

The general inclusion criteria for Chapter 4, 5 and 6 were as follow:
* Normal vision (with and without glasses) for neuropsychological testing
* Normal hearing range (with or without hearing aid) for neuropsychological testing
* Fluent English (due to the nature of the neuropsychological tests)
* Willing and able to participate in neuroimaging study (repeated MRIs at 3 Tesla in one session)

An additional inclusion criterion for Chapter 4 was 'stability of permitted medications for 4 weeks'. The decision for the inclusion of individuals taking medication is addressed in chapter 4's discussion as well as in the final discussion (Chapter 7).

The general exclusion criteria for Chapter 4, 5 and 6 included:
* MRI screening form [Briefly they include: claustrophobia, active medical implants, passive implants deemed unsuitable, pregnancy or possible pregnancy; previous experience with metalworking without eye protection]
* Presence of pacemakers, shrapnel, or other metal implants/objects in the eyes, skin, or body
* Claustrophobic
* Cerebrovascular disease
* Ischaemic heart disease
* Depression
* Psychiatric diseases
* History of stroke or seizure

∗ Hospital Anxiety and Depression Scale – depression cut-off score ≥8 – anxiety cut-off score ≥10

∗ Mini Mental State Examination – cut-off score ≤23

A further exclusion criterion for Chapter 5 and 6 covered 'use of prescription and non-prescription medication', which is covered in the general discussion in the final Chapter 7. All inclusion and exclusion information provided by the participants were self-reported.

All potential participants received study information and had the opportunity to ask questions to make an informed choice. After agreeing to take part, all participants provided written informed consent (Appendix F and G) and were debriefed at the end of the study with the opportunity to ask more questions (Appendix H). All data were collected in one visit.

2.4. Neuropsychological assessment

The neuropsychological assessment battery was administered by myself in accordance with procedures described in the relevant manuals. The testing took place in a quiet, separate room prior to the scanning session. These tests were only administered to the study cohort used in Chapter 5 and 6.

Individuals were screened for depression and anxiety with the help of the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983). The HADS is divided into two subscales, depression and anxiety, with 7-items each, while the score for each subscale ranges from 0 to 21. The HADS assumes a score between 0-7 as normal, 8-10 as mild, 11-14 as moderate, and 15-21 as severe. Depression may impact on the neurochemical composition of the brain (Yildiz-Yesiloglu & Ankerst, 2006); as a result, individuals with mild to high depression were excluded from the studies. Leeway was given for anxiety scores (cut-off point ≥10), as the testing and scanning session might have contributed to anxiety.

General cognition was assessed with the Mini Mental State Examination (MMSE, Folstein, Folstein & McHugh, 1975). It is a well-established screening tool for cognitive abilities with a good reliability and validity (Tombaugh & McIntyre, 1992). It assesses orientation to time, orientation to place, registration, attention and calculation, recall, naming, repetition, comprehension, reading, writing, and drawing. All participants scored (younger: $M \pm SD =$ 28.2 ± 1.7; older: $M \pm SD =$ 28.7 ± 1.5) within the recommended normal range for cognition on the MMSE (cut-off score ≤23).

The Stroop Neuropsychological Screening Test (SNST, Trenerry, Crosson, DeBoe, & Leber, 1989) assesses the ability to inhibit unwanted interference from incongruent colour words. In the first instance a participant is asked to read words (congruent colour words), which represent the same colour they are printed in, as quickly and accurately as possible in two minutes. Subsequently, the participant is asked to name the colour of the words (incongruent colour word), which are printed in a different colour to what they represent, in two minutes. Individuals who struggle with naming the colour of the colour-words will experience the Stroop Interference effect. Previous research has reported of an increased reaction time and error rate on naming the incongruent colour words in older compared to younger individuals (Davidson, Zacks, & Williams, 2003).

Tests of immediate and delayed verbal and visual memory were used to determine any age-related changes in cognitive functions with the help of the Wechsler Memory Scale – Third Edition (WMS-III, Wechsler, 1997a, 1997b) subtests: Logical Memory and Visual Reproduction. The Logical Memory test measures immediate and delayed recall of two short stories. After auditory presentation of each short story the participant is asked to recall it from memory immediately after hearing. Subsequently, the participant is asked to remember the stories and to recall them after 35 minutes. The participant is scored on the accuracy of recall of each story. The immediate and delayed scores represent the information remembered by the participant. The Visual Reproduction test measures immediate and delayed recall of geometric designs. After 10 seconds of visual presentation of a geometric design the participant is asked to draw it from memory. All together five geometric designs are presented and the participant is asked again to remember all of them. After 35 minutes the participant is asked to draw all five designs from memory. The participant is scored on the accuracy of drawing each design. Similarly, the scores for immediate and delayed recall serve as measures for remembered information by the participant.

Chapter 3

A systematic review of ^{1}H metabolite T$_2$ relaxation time in healthy human brain

3.1. Abstract

Transverse relaxation (T$_2$) time measurement of neurometabolites can provide important information about the cellular environment and is essential for metabolite quantification. Specifically, the lack of appropriate correction for T$_2$ changes may introduce errors in metabolite concentrations leading to incorrect interpretations of concentration changes in disease. This systematic review provides an overview of proton metabolite T$_2$ values in brain tissue, as well as addressing possible difficulties regarding T$_2$ research and proposing consensus T$_2$ values for neurometabolites in grey matter, white matter, and mixed tissue content. Literature was searched using the terms "magnetic resonance spectroscopy" combined with "T2" or "transverse relaxation". The review identified 46 studies matching the inclusion criteria. Due to limited data for Glu and MI only NAA, Cr and Cho T$_2$ relaxation times at 1.5T and 3T were considered for further investigation. The outcome suggests that NAA T$_2$ values show a strong separation between grey matter, white matter, and mixed tissue at 1.5T and 3T, while Cr T$_2$ values displayed differences between grey and white matter at 3T, and Cho T$_2$ values showed a significant difference between grey matter and mixed tissue at 1.5T. While clear consensus values for T$_2$ relaxation arise from this review, potential methodological challenges performing T$_2$ measures, such as echo time, J-coupling, and exponential fit, can introduce uncertainties and are discussed here.

Keywords: T2, transverse relaxation, proton magnetic resonance spectroscopy, metabolites, systematic review

3.2. Introduction

Recent review publications show a growing interest in the use of proton magnetic resonance spectroscopy (^{1}H-MRS) in healthy ageing and disease (Haga et al., 2009; Tumati et al., 2013). Particularly as metabolic changes can provide important information regarding early degeneration or disease progression without the use of brain tissue biopsy (Haga et al., 2009; Kantaric, 2013). ^{1}H-MRS is a non-invasive in vivo imaging technique, which allows direct acquisition of brain metabolites (Ross & Bluml, 2001). The process by which brain metabolites are quantified is important in determining the absolute concentration levels of metabolites of interest (Ross & Bluml, 2001; Osorio-Garcia et al., 2012). One of the quantification factors to consider is transverse relaxation, known by other synonyms such as spin-spin and T$_2$ relaxation time (Buxton, 2009; Huettel et al., 2009). Nuclear spins align in the x-y plane after a 90° radio frequency (RF) pulse when transverse magnetization is at its highest, and subsequently fall out of phase resulting in signal lose. This can occur due to coupling with other spins (spin-spin relaxation), referred to as intrinsic transverse relaxation (T$_2$), or due to a combination of spin-spin relaxation and magnetic field inhomogeneity, denoted as apparent transverse relaxation (T$_2$*). Equally important, T$_2$ relaxation shortens as molecular motion decreases. To compensate for the resulting signal loss, often a 180° RF pulse is applied to refocus the nuclear spins resulting in phase coherence and producing a signal at two times the time between the 90º and the 180º pulses (Hahn, 1950). ^{1}H-MRS typically utilises the spin echo effect to both reduce T$_2$* effects and localise signal acquisition to a defined region of interest (ROI) via either a train of three slice selective 90º pulses orthogonal to each other (Stimulated Echo Acquisition Method, STEAM) (Frahm et al., 1989) or a train of one slice selective 90º pulse followed by two slice selective 180º pulses (Point RESolved Spectroscopy, PRESS) (Bottomley, 1987), while both these techniques reduce the effect of T$_2$* decay on the signal, the signal generated is still affected by T$_2$ relaxation.

The T$_2$ relaxation of a metabolite is a time constant, which is measured by acquiring metabolic signals at different echo-times (TE) and fitting them to an exponential decay curve (Huettel et al., 2004; Whittall, MacKay, & Li, 1999). This can be determined by using a mono- or multi-exponential function depending on the metabolite's environmental behaviour. The exponential decay function is defined as

$$S(TE) = S(0) * e^{-TE/T2}$$

where S(TE) represents the signal at a given TE, and S(0) corresponds to the signal at TE=0 (Rutgers & Van der Grond, 2002). The estimated T_2 relaxation values are apparent T_2 values, as T_2^* decay cannot fully be eliminated. However, the term T_2 relaxation is more commonly used in the literature and we will continue using T_2. Research in this field documents that each metabolite has a specific relaxation time, which decreases with increased magnetic field strength (Li et al., 2013).

The three main metabolites commonly studied using ^{1}H-MRS are N-Acetyl Aspartate (NAA), creatine (Cr, the sum of creatine and phosphocreatine) and choline (Cho, the sum of choline, phosphocholine and glycero-phosphocholine), all of which give rise to singlet signals in ^{1}H-MRS (Rae, 2014). These metabolites are those with the most extensive research regarding T_2 relaxation due to the ease of characterising signal changes in the large singlet peaks present (Rutgers & Van der Grond, 2002). Other metabolites such as the functional markers glutamine (Gln), glutamate (Glu), gamma-aminobutyric acid (GABA) and myo-Inositol (MI) are scalar coupled and give rise to complex multiplets in the ^{1}H-MRS spectrum (Edden, Intrapiromkul, Zhu, Cheng, & Barker, 2012; Ganji et al., 2012). This creates a problem as the spectral peaks for scalar coupled metabolites are not only affected by T_2 relaxation but also by coupling (J-coupling), which needs to be considered when attempting to measure T_2 relaxation values (Ganji et al., 2012). Not only do T_2 measures provide information regarding the local environment of metabolites of interest but also play a crucial role in estimation of metabolite concentrations from ^{1}H-MRS data. Metabolite concentrations in ^{1}H-MRS data are often arrived at through comparison to a reference signal from another chemical of known or assumed concentration (Gasparovic et al. 2006; Henning et al., 1992). Concentration referencing is based on the fact that the amount of signal obtained from a metabolite in ^{1}H-MRS is proportional to the concentration of the metabolite. This gives rise to a simplistic model such that $Signal_{(A)}/Signal_{(B)} \propto Concentration_{(A)}/Concentration_{(B)}$. However, T_2 relaxation also influences signal, and as metabolites of interest often have different T_2 relaxation times, the effect of T_2 relaxation needs to be accounted for appropriately. Failure to account for T_2 correctly can lead to substantial errors, which can lead to confounding results if incorrectly performed (Barker et al.,1993; Rutgers & Van der Grond, 2002). A lack of awareness of potential T_2 changes may also lead to incorrect interpretation of concentration changes in disease. For this reason, several researchers have measured the T_2 of several metabolites, with the results readily found in the literature (Rutgers & Van der Grond, 2002). As such there exist several reports of T_2 relaxation times for the main metabolites NAA, Cr and Cho, as well as a few for other metabolites such as glutamate, but it is not clear which of these measures, if any, is definitive. Likewise there is mixed information regarding how a

metabolite's literature T$_2$ value differs between regions containing mostly grey matter (GM), mostly white matter (WM), or both and how this may change across age.

The purpose of this review therefore was: 1) to provide an overview of the existing research investigating neurometabolite T$_2$ relaxation times using ^{1}H-MRS in the human brain; 2) to identify and discuss some of the possible obstacles associated with T$_2$ measurements in research; and 3) where possible to present a consensus T$_2$ value for NAA, Cr, Cho, MI and Glu in regions of GM, WM and mixed tissue content. It is hoped that the values so obtained will be of use to fellow researchers using ^{1}H-MRS in their research.

3.3. Methods

3.3.1. Procedure

On 3rd December 2014 and updated in August 2015, a literature search was performed in the PubMed and ProQuest databases. The following key terms were used for the search: "magnetic resonance spectroscopy" combined with "T$_2$ relaxation" or "transverse relaxation". The inclusion criteria used in this study selection were as follow:

- The article has been written in English and published in a peer-reviewed journal.

- The study utilised proton magnetic resonance spectroscopy as a measurement tool.

- The study population included healthy humans.

- Metabolite data were acquired in vivo in the human brain.
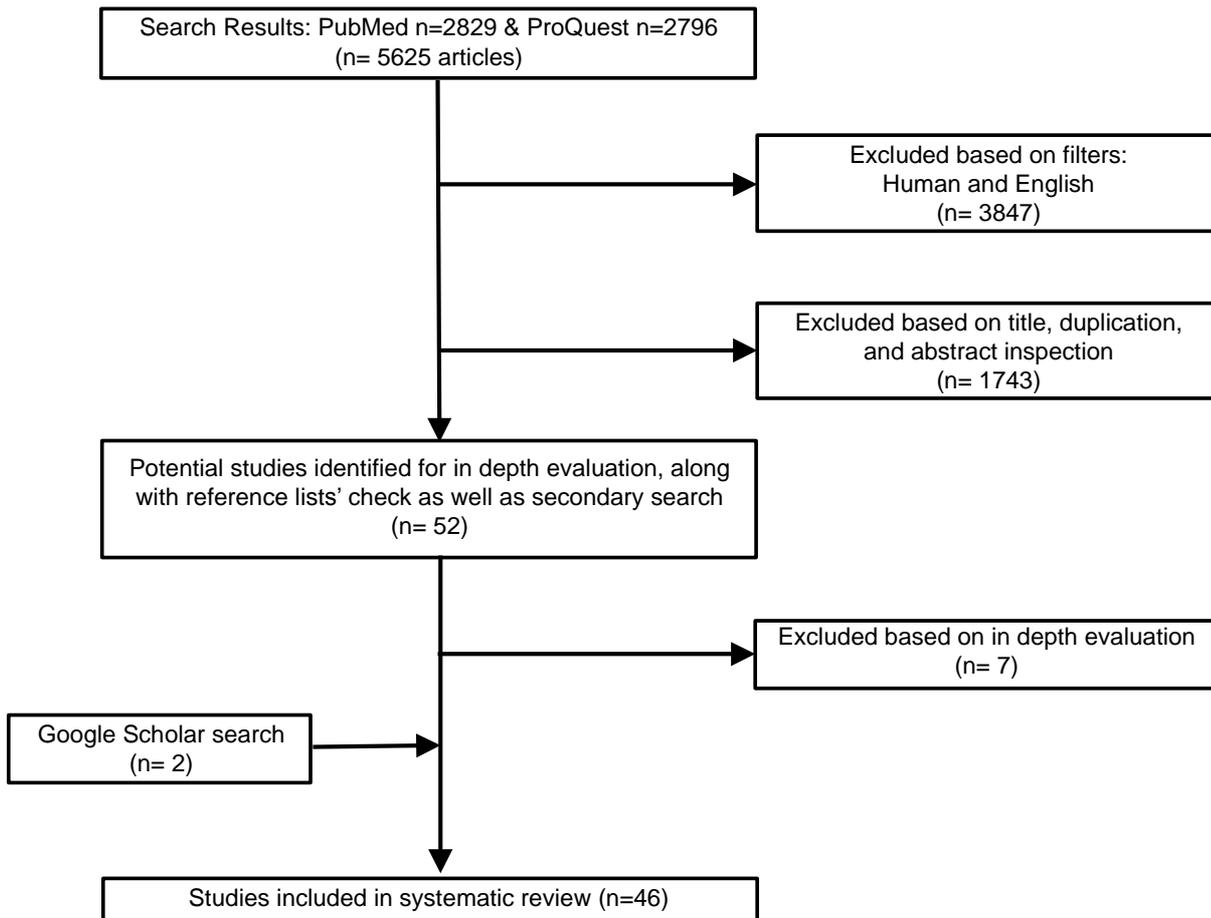
Figure 1. Study selection process representing the total number of identified articles.

In line with the above outlined search terms a total of 5,625 papers were identified (Figure 1). Subsequently, filters (human and English) were applied to titles and abstracts and 3,847 papers were rejected as a result of failing to meet the inclusion criteria. After closely examining titles and abstracts, based on the above inclusion criteria, 34 papers were retrieved for detailed inspection and examination of reference lists for additional relevant studies. Ten additional papers were found in the reference lists of the 34 included papers as well as two papers (Brooks et al., 2001; Li, Xu, Ozturk-Isik, Lupo, Chen, Vigneron, & Nelson, 2013) found in Google Scholar. Two review studies (Kreis, 1997; Henriksen, 1995) were excluded as our search returned the same papers of interest. In addition, a study (Hanstock, Rothman, Prichard, Jue, & Shulman, 1988) was excluded based on the use of surface coil rather than a birdcage coil, which was not comparable to the other studies that used birdcage coils. The authors reported NAA and Cr T$_2$ values at 1cm and 4cm depth from the skull along

with varying data points used to determine each T$_2$ value (Hanstock, Rothman, Prichard, Jue, & Shulman, 1988).

In each of the identified articles, the authors' classification of GM has been adapted in this chapter, whereas voxels which the authors have not clearly defined the tissue content are considered to consist of mixed tissue, both GM and WM.

3.3.2. Statistical analysis

A one-way between-groups analysis of variance (ANOVA) will be conducted to examine differences of mean metabolite T$_2$ relaxation times across brain tissue (WM, GM, and mixed tissue). In addition, forest plots were used to display NAA, Cr, and Cho at 1.5T and 3T, while Glu T$_2$ values were displayed at 3T, 4T, and 7T in one forest plot.

3.4 Results

Altogether 46 studies met the inclusion criteria and are summarised in Table 1. The studies were arranged according to magnetic field strength and further organized by date of publication. Data extraction included, where available: participant total (N); age; gender; region(s) of interest (with voxel size); MRS sequence; metabolite(s) of interest; echo time (TE) and repetition time (TR). The identified studies combined a total of 779 participants with varying age from postnatal up to and including old age. Information about gender was not available from all of the studies. TE and TR ranged from 1ms to 1500ms and 450ms to 12000ms, respectively, as well as the number of echoes used per study ranged from 2 to 64. Information on predominantly implemented magnetic field and ^{1}H-MRS sequence are provided in Table 2.

Table 1. Summary of papers included in systematic review

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
1.5 Tesla							
Malucelli et al., 2009	WM: 15 participants (5) GM: 15 participants (5)	Mean: 39 SD=16 Mena: 36 SD=15	mid-brain parietal-occipital cortex (3 x 3 x 2) left-parietal-occipital white matter (2 x 2 x 2)	PRESS	NAA, Cho, Cr	35, 70, 100, 144, 288	4000
Kreis et al., 2005	25 participants	Range: 15 - 78	centrum semiovale (10 - 16 cm^3)	PRESS	NAA(3), mI, GPC(3), Cr(3), Cr(2)	20, 30, 40, 62.5, 100, 200, 500, 1500	12000
Soher et al., 2005	6 participants	Range: 5 - 45	parieto-occipital lobe mixed gray-white matter (8cm^3)	CP-PRESS	NAA. Cr, Cho, mIns	26, 66, 106, 146, 186, 226	1500
Brief et al., 2005	occipital GM: 10 participants (6) posterior frontal WM: 10 participants (4)	Mean: 29 SD=9 Mean: 31 SD=13	occipital GM (1.93 x 1.93 x 2) posterior frontal WM (1.93 x 1.93 x 2)	PRESS	NAA, Cr, Cho	30, 60, 100, 150, 200, 400, 600, 800	2000
Kugel et al., 2003	84 infants	Mean: 37.8 weeks SD=2.2	basal ganglia size: 2.9 mL, SD=0.8	PRESS	Cho, Cr, NAA, MI, Lac	25, 136, 272	1886 6000

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm³ or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Ke et al., 2002	26 participants (11)	Mean: 34 SD=6	left anterior frontal lobe (2.5 x 2.5 x 2)	PRESS	PCr, Cr	48 - 678 (in 10-ms increments)	2640
Mascalchi et al., 2002	38 participants (18)	Mean: 34	midbrain, pons, dentate and vermis (2 x 2 x 2)	PRESS	NAA, Cho, Cr	80, 136, 272, 400	2000
Rutgers et al., 2002	15 participants (7)	Mean: 21 SD=2	centrum semi-ovale of a cerebral hemisphere, WM only (Mean: 14cm3, SD=4.3)	PRESS	NAA, Cho, Cr	35, 60, 120, 170, 288, 408, 588, 864	2250
Brooks et al., 2001	50 participants	Mean: 45.5	interhemispheric fissure of the medial frontal lobe (2 x 2 x 2)	STEAM	NAA, Cho, Cr	72, 144, 216, 288	2971.3
Ala-Korpela et al., 1995	19 participants	Mean: 27	frontal, parietal, occipital, temporal, thalamic and cerebellar (2 x 2 x 2)	PRESS	NAA, Cho, Cr	60, 270	1500
Longo et al., 1995	12 younger participants (8) 6 older participants (5)	age: 28 age: 76	temporal-parietal region of the right hemisphere (3 x 3 x 3) GM/WM	PRESS	NAA, Cho, Cr	50, 136, 272	4500

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Danielsen et al., 1994	12 participants (7)	Mean: 27 SD=5	occipital lobe primarily WM (2 x 2 x 2)	STEAM	NAA, Cho, Cr	20, 46, 92, 136, 272, 544	2000
Toft et al., 1994	22 infants	Range: 0 - 48 weeks	2 x 2 x 2 to cover most of the caudate nucleus, putamen, and globus pallidus and 1 voxel in the occipital lobe	STEAM	NAA, Cho, Cr	20, 46, 92, 272	1600
Barker et al., 1993	10 participants (3)	Mean: 34 SD=9	frontal lobe white matter (27cm3) and thalamus (8cm3)	STEAM	NAA, Cho, Cr	30, 60, 90, 120, 200, 270	1000 3000 1500
Christiansen et al., 1993	5 participants		occipital lobe (27, 15.6, and 8 ml)	STEAM	NAA, Cho, Cr	46, 92, 136, 272	1500
Toft et al., 1993	8 neonates 8 adolescents	Range: 259 - 2095 days Range: 10 -15 years	Neonates area: caudate nucleus, putamen, and globus pallidus (2 x 2 x 2) Adolescent 4 areas: occipital lobe, basal ganglia, temporal lobe, and frontal lobe (2 x 2 x 2)	STEAM	NAA, Cho, Cr, Ins (glycine)	20, 46, 92, (272 not included as low SNR)	1600

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Kreis, Ernst & Ross, 1993	6 Newborns 7 Adults	Mean: 42 weeks SD=3 Mean: 1460 weeks SD=83	midline occipital cortex left or right parietol-occipital lobe voxel size in very young babies were between 3 and 8 cm, and 8 to 16 in older children	STEAM	NAA. Cr, Cho, mI	30, 40, 60, 90, 135, 270	1500 - 5000
Kreis, Ernst & Ross, 1993	11 participants (7)	Range: 23 - 33	posterior parietal cortex and occipital cortex	STEAM	NAA. Cr, Cho, mI	30 (twice), 40, 60, 90, 135, 270 (twice)	2870
Christiansen et al., 1993	8 participants 8 participants	Mean=24.9 Mean=67.3	occipital, basal ganglia, temporal, and frontal (2 x 2 x 2)	STEAM	NAA, Cho, Cr	20, 46, 92, 272	1600
Henning et al., 1992	34 participants (15)	Range: 25 - 28	parietal (32), occipital (25), frontal (21), and cerebellum (27) (2 x 2 x 2)	PRESS	NAA, Cho, Cr	52, 60, 70, 85, 100, 115, 130, 150, 175, 200, 225, 250, 275, 300, 350, 400, 500, 600, 800, 1000	5000
Narayana et al., 1991	6 participants	Range: 24-35	frontal lobe 27cc	STEM	NAA, Cho, Cr	20, 35, 50, 75, 100	4000

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Frahm et al., 1989	14 participants (0)	Range: 20 - 35	occipital area (4 x 4 x 4), occipital lobe (4 x 4 x 4), insular area (3 x 3 x 3), thalamus (3 x 3 x 3), cerebellum (4 x 4 x 4)	STEAM	Lac, NAA, tCr, Cho, Ins	50, 135, 270	1500
2 Tesla							
Choi & Frahm, 1999	8 participants		bilateral hippocampal areas (1.3 x 1.8 x 2.3)	STEAM	tNAA, tCr, Cho, Ins	20, 135, 270	1500 3000 6000
2.4 Tesla							
Cady et al., 1996	27 infants	1 - 46 days	thalamic region in 19 infants and occipito-parietal region in 12 infants	PRESS	NAA, Cr, Cho, Lac	135, 270, 540	1730
3 Tesla							
Edden et al., 2012	5 participants (3)	Mean: 39.9 SD=7.9	occipital lobe (3 x 3 x 3)	MEGA-PRESS	NAA, Cr, Cho, GABA	70, 100, 180	2000
Ganji et al., 2012	5 participants (2)	Mean: 27 SD=7	medial occipital GM and left occipital WM (25 x 30 x 30)	PRESS	NAA, Cr, Cho, MI, Glu	54, 112, 246, 374	3000

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Kirov et al., 2008	4 adolescents (3) 8 young adults (4) 2 middle-aged (1) 6 elderly (3)	Mean: 13 SD=1 Mean: 26 SD=1 Mean: 51 SD=6 Mean: 74 SD=3	Multivoxel 10cm anterior-posterior X 8cm left -right X 4cm inferior-superior = 320cm3	PRESS	NAA, Cr, Cho	35, 260	1260
Zaaraoui et al., 2007	8 participants (4)	Mean: 26	Multivoxel 10cm anterior-posterior X 8cm left -right X 4cm inferior-superior	PRESS	NAA, Cr, Cho	35, 285	1000
Tsai et al., 2007	6 participants (3)	Mean: 30 SD=10	(2 x 2 x 2)	PRESS PEPSI	NAA, Cr, Cho	50, 100, 160, 220, 300	1200
Choi et al., 2006	5 participants	Mean: 20-30	medial PF cortex and LF brain (3 x 2.5 x 2.5)	PRESS	Glu, tCr	128, 164, 214, 262, 326, 380	3000 3037 3089 3140 3208 3267
Schubert et al., 2004	3 participants		anterior cingulate cortex (2.5 x 4 x 2) and left hippocampus (2 x 3 x 2)	PRESS	Glu, NAA, tCr, tCho	50, 80, 135, 250, 330	3000

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Hurd et al., 2004	6 participants (3)	Range: 22-52	mid-parietal 'mostly' GM and left parietal 'mostly' WM (8 cc)	TE-Averaged PRESS	NAA, Cr, Cho	35-335	2000
Traber et al., 2004	42 participants (20)	Mean: 40 SD=18	3T Occipital WM (N=10) Motor cortex GM (N=10) Cingulate gyrus GM (N=6) Frontol.WM/GM (N=7) Basal gnaglia (N=8) Cerebellum (N=5) 1.5T Occipital WM (N=15) Motor cortex GM (N=8) Frontol WM/GM (N=5)	PRESS	NAA, tCr, tCho	50, 120, 200, 280, 400	2500
Mlynarik et al., 2003	8 participants (5)	Mean: 30	GM or WM in occipital lobe (2 x 2 x 2)	STEAM	NAA, Glu, Cr, Cho, MI, Glx	50, 100, 150, 200, 250	6000
4 Tesla							
Marjanska et al. (2013)	18 young participants (12) 14 older participants (7)	Mean: 20 SD=1 Mean: 77 SD=5	occipital lobe (3 x 3 x 3)	STEAM	NAA, tCr, and tCho	10, 20, 30, 40, 60, 80, 180	450 (mixing time of 42ms)

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Deelchand et al., 2012	6 participants	Mean: 37 SD=17	visual cortex (3 x 3 x 3)	LASER	NAA, tCr, tCho, Glu, Ins, Tau	53, 75, 100, 150, 200, 300, 400	4000
Posse et al., 1995	16 participants		occipital lobe and temporal regions (2 x 2 x 2)		Cho, Cr, NAA	20, 30, 50, 100, 150, 200, 300, 400	4000
4.1 Tesla							
Mason et al., 1995	8 participants				Cho, tCr, NAA		
Hetherington et al., 1994	5 participants		1.15cc	STEAM	NAA, Cr, Cho	TE: 50, 100, 150, 200, 250 TIR: 45, 125, 225, 325, 425	2000
7 Tesla							
Ronen at al., 2013	9 participants (6)	Mean: 25 SD=4	**Protocol A:** medial parietal GM (2 x 2 x 2)	PRESS	tCr, tCho, NAA	40, 110, 180	4000
			Protocol B: 1 x parietal WM 1 x parietal GM (2 x 2 x 2)			40, 180	
Intrapiromkul et al., 2013	5 participants (4)	Mean: 26.6 SD=3.1	posterior cingulate cortex 3 x 3 x 3	MEGA-PRESS (with MM suppression)	GABA	70, 90, 110	3000

Study Reference	Participants n (female)	Age (mean, median or range) SD	ROI (voxel size [cm^3 or ml])	MRS sequence	Metabolites	TE (ms)	TR (ms)
Marjanska et al., 2012	23 participants (11)	Mean: 23 SD=4	occipital lobe (2.7 x 2.7 x 2.7) motor cortex (2 x 2 x 2) basal ganglia (1.4 x 4 x 1.5) cerebellum (2.5 x 2.5 x 2.5)	LASER	NAA. tCr, tCho, Glu, GHS, mIns, sIns	35, 70, 105, 140, 175, 210	2000
Tkáč et al., 2001	18 participants		occipital lobe (2 x 2 x 2)	STEAM	NAA, tCr	10, 20, 30, 40, 60, 80, 100, 140, 180, 250	5000
Double studies							
Barker et al., 2001	5 participants (2)	Mean: 37 SD=5	Voxel selected at random in the left or right centrum semiovale (2 x 2 x 2)	STEAM	Water, Cho, Cr, NAA	20, 67, 136, 272	3000
Michaeli et al., 2002			visual cortex (2 x 2 x 2)	PRESS CP-LASER	Water, NAA, Cr	4T, five TEs from 50 to 170ms, and 7T, from 30 to 130, for PRESS\n\n4T and 7T, from 37.8 to 138.6, for CP-LASER	3000 4500

Magnetic field strength	1.5T	2T	2.4T	3T	4T	4.1T	7T	Other
No. of studies	23	1	1	10	3	2	4	3

MRS sequence	PRESS	STEAM	LASER	MEGA-PRESS	Other			
No. of studies	22	18	3	2	2			

Table 2. Summary of predominantly implemented magnetic field strength and ^{1}H-MRS sequence. Following acquisition sequences were used: Point RESolved Spectroscopy sequence (PRESS); Stimulated Echo Acquisition Mode (STEAM); Localization by Adiabatic Selective Refocusing (LASER); MEscher-GArwoods Point RESolved Spectroscopy (MEGA-PRESS); and two studies used other sequences (Proton Echo Planar Spectroscopic Imaging (PEPSI) and Carr-Purcell Point RESolved Spectroscopy (CP-PRESS)).

Forest plots were used to display NAA (Fig. 2, Fig. 3), Cr (Fig. 4, Fig. 5), and Cho (Fig. 6, Fig. 7) only at 1.5 T and 3T and Glu (Fig. 8) at 3T, 4T and 7T due to limited data at other magnetic field strengths. As expected, the review finds strong empirical support for T$_2$ relaxation time decreasing with increasing magnetic field strength.
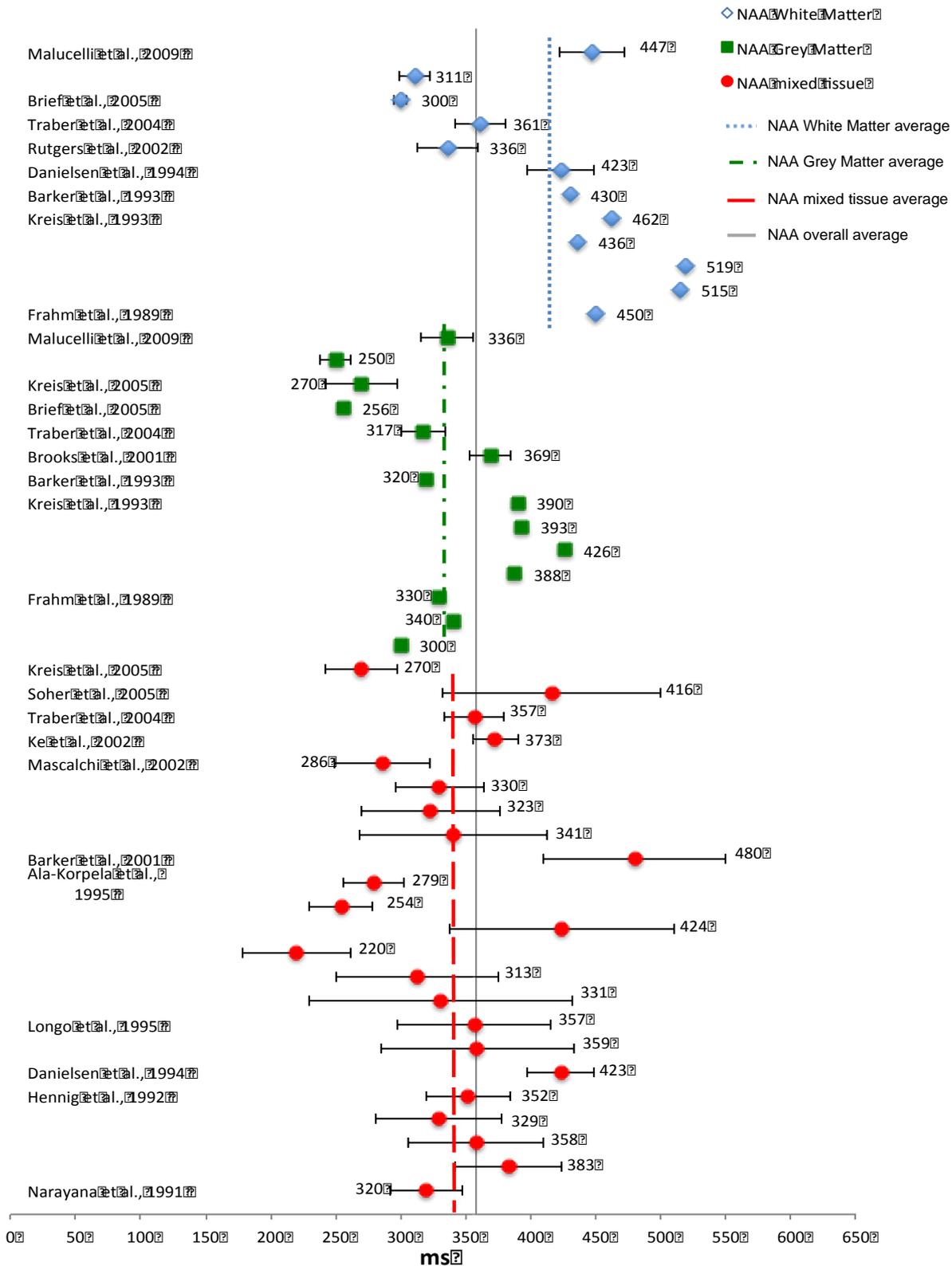
NAA at 1.5T

Figure 2. Illustrated are NAA T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 1.5T with a 95% CI. A grey line displays the overall mean at 358ms, while the blue, red and green lines display the mean T$_2$ values of WM at 416ms, GM at 335ms, and mixed tissue at 343ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.

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Figure 3. Illustrated are NAA T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 3T with a 95% CI. A grey line displays the overall mean at 300ms, while the blue, red and green lines display the mean T$_2$ values of WM at 343ms, GM at 268ms, and mixed tissue at 249ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.

NAA at 3T
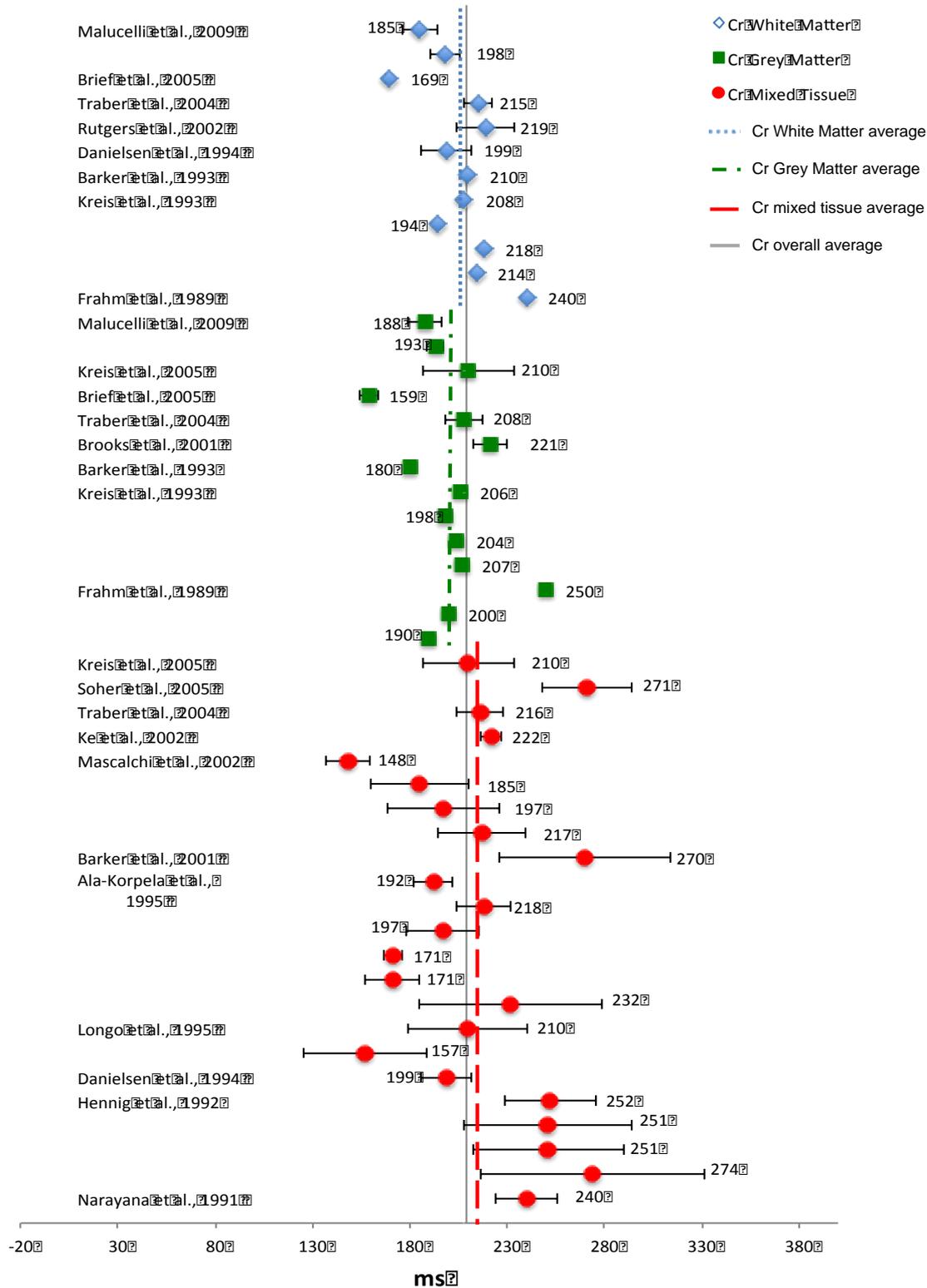
Cr at 1.5T

Figure 4. Illustrated are Cr T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 1.5T with a 95% CI. A grey line displays the overall mean at 209ms, while the blue, red and green lines display the mean T$_2$ values of WM at 206ms, GM at 201ms, and mixed tissue at 215ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.
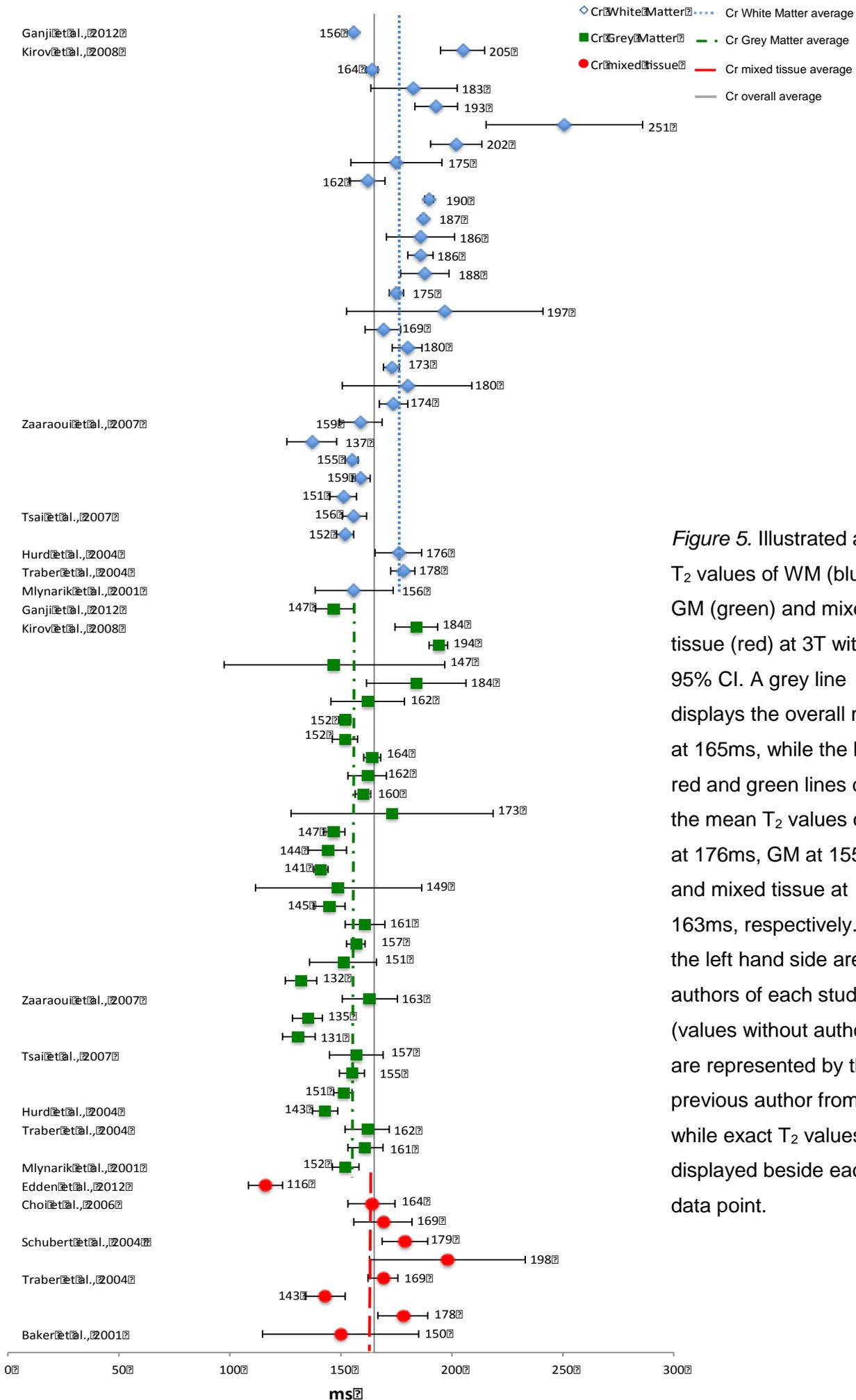
Figure 5. Illustrated are Cr T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 3T with a 95% CI. A grey line displays the overall mean at 165ms, while the blue, red and green lines display the mean T$_2$ values of WM at 176ms, GM at 155ms, and mixed tissue at 163ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.

Cr at 3T
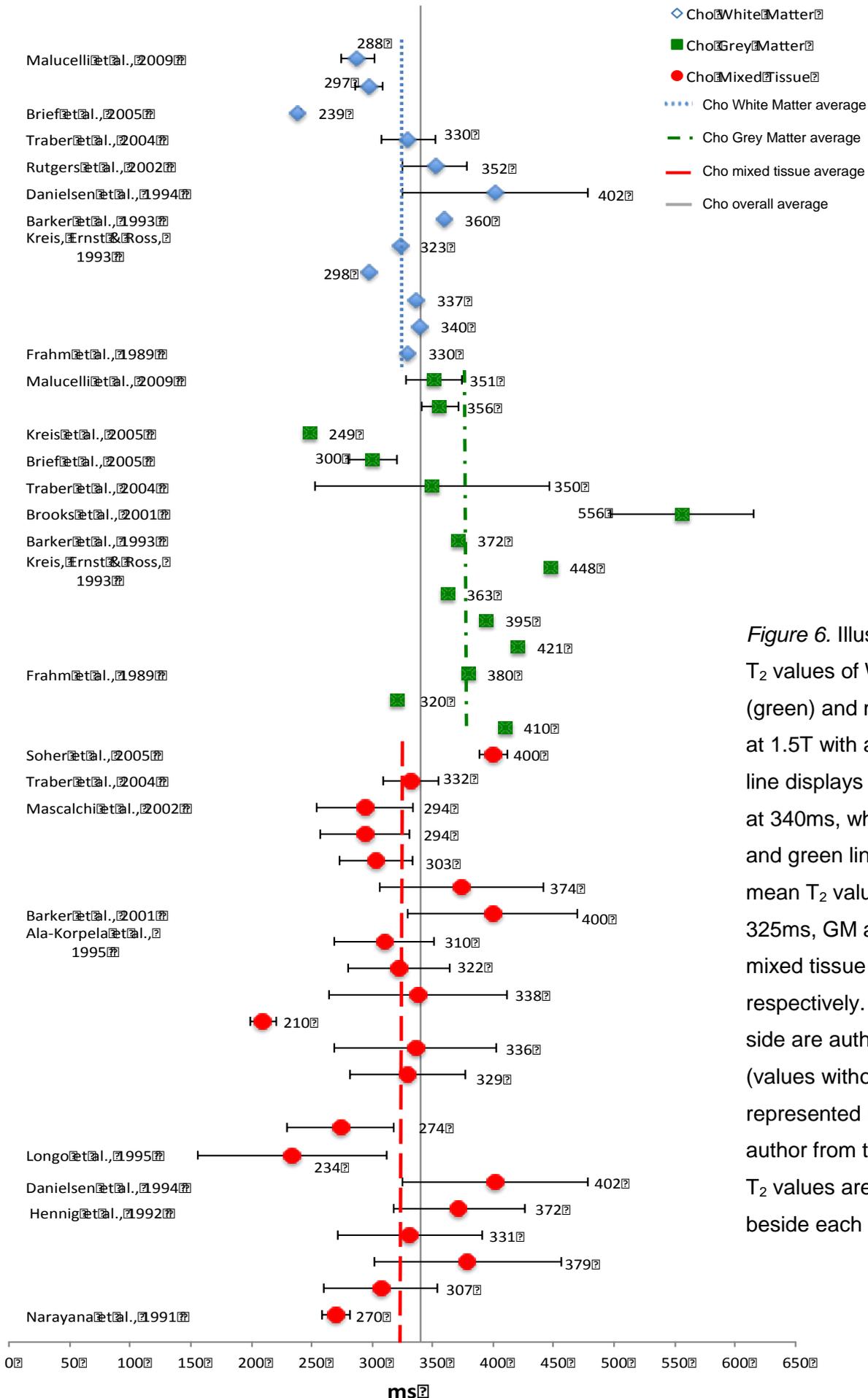
Figure 6. Illustrated are Cho T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 1.5T with a 95% CI. A grey line displays the overall mean at 340ms, while the blue, red and green lines display the mean T$_2$ values of WM at 325ms, GM at 377ms, and mixed tissue at 324ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.

Cho at 1.5T
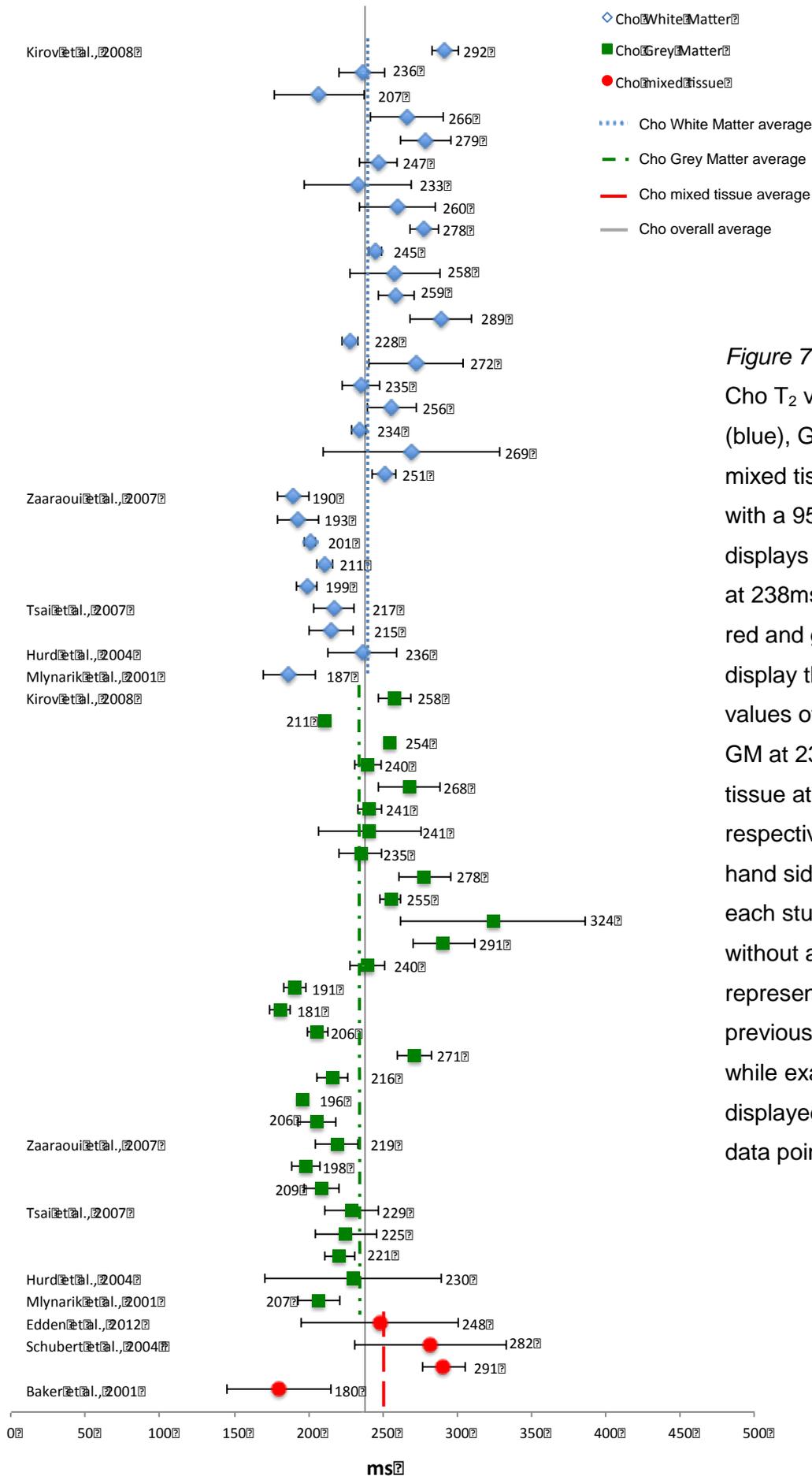
Figure 7. Illustrated are Cho T$_2$ values of WM (blue), GM (green) and mixed tissue (red) at 3T with a 95% CI. A grey line displays the overall mean at 238ms, while the blue, red and green lines display the mean T$_2$ values of WM at 239ms, GM at 234ms, and mixed tissue at 250ms, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T$_2$ values are displayed beside each data point.

Cho at 3T

Glu

◆ Glu at 3T   ■ Glu at 4T   ● Glu at 7T
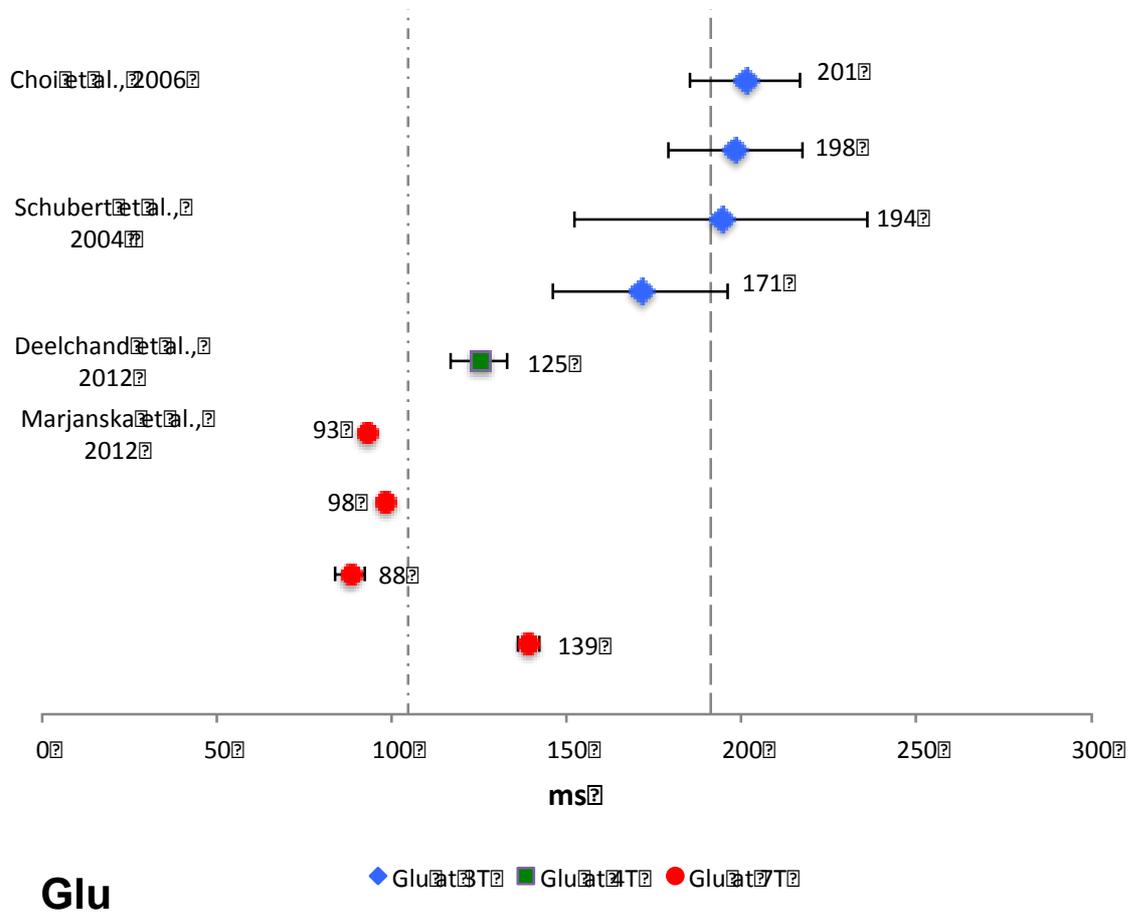
Figure 8. Illustrated are Glu T_2 values of WM/GM at 3T (blue), 4T (green), and 7T (red) with a 95% CI. The overall mean is displayed at 191ms and 104.5ms for 3T and 7T, respectively. On the left hand side are authors of each study (values without authors are represented by the previous author from top), while exact T_2 values are displayed beside each data point.

Overall, mean T_2 relaxation values for NAA, Cr, Cho, and Glu are summarised by brain tissue in Table 3. A one-way between-groups analysis of variance was conducted to explore differences of NAA, Cr, and Cho T_2 relaxation times in brain tissues (Group 1: WM; Group 2: GM; Group 3: mixed tissue) at 1.5T and 3T. NAA T_2 relaxation at 1.5T was significantly different between the groups: $F(2, 46) = 6.97$, $p = .002$. Post-hoc comparison using Bonferroni test indicated that the mean score for WM ($M = 416$, $SD = 73.36$) was significantly different from GM ($M = 335$, $SD = 54.17$) and mixed tissue ($M = 342$, $SD = 59.76$). There was no significant difference between NAA T_2 values for GM and mixed tissue. Similarly there

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was a statistically significant difference between NAA T$_2$ relaxation times at 3T for the three tissue groups: $F(2, 67) = 14$, $p < .001$. The post-hoc test determined a significant difference between WM ($M = 346$, $SD = 68.63$) and GM ($M = 268$, $SD = 63.51$) as well as WM and mixed tissue ($M = 249$, $SD = 37.45$). In addition, Cr T$_2$ relaxation times at 3T were significantly different ($F(2, 69) = 7.66$, $p = .001$), where Bonferroni test showed a significant difference between WM ($M = 175$, $SD = 21.68$) and GM ($M = 156$, $SD = 14.74$). Mixed tissue ($M = 163$, $SD = 23.87$) did not differ significantly from either WM or GM. As well as no significant changes were observed for Cr T$_2$ relaxation times at 1.5T. Cho T$_2$ relaxation values at 1.5T showed a significant difference between groups ($F(2, 44) = 4.13$, $p = .02$). Post-hoc comparison using the Bonferroni test indicated that the mean score for GM ($M = 377$, $SD = 72.35$) was significantly different from mixed tissue ($M = 324$, $SD = 52.52$), while there was no significant difference between WM ($M = 325$, $SD = 41.10$) with GM and mixed tissue. There were no significant differences between Cho T$_2$ relaxation values for the three brain tissue groups at 3T.

		NAA	SD	Cr	SD	Cho	SD	Glu	SD
1.5T	**WM**	415	73	206	18	325	41		
	GM	335	54	201	21	377	72		
	Mixed	343	60	215	36	324	53		
	Overall	358	69	209	29	340	61		
3T	**WM**	346	69	175	22	237	33		
	GM	268	64	156	15	234	33		
	Mixed	249	37	163	24	250	50	191	14
	Overall	302	75	166	21	236	34		

Table 3. T$_2$ mean values (from included studies in systematic review) for ^{1}H-MRS metabolites at 1.5T and 3T by brain tissue content (M values and SD in ms).

3.5. Discussion

3.5.1. Systematic review literature summary

T$_2$ relaxation measures are important part of metabolite quantification, while also providing information regarding cellular environment and pathological changes in ageing and disease (Ross & Bluml, 2001; Frahm et al., 1989; Öngür et al., 2010). The first aim of this review was to present the most up to date list of in vivo neurometabolite T$_2$ relaxation times implementing ^{1}H-MRS limited to the human brain. Overall, 46 studies were identified matching the inclusion criteria for this review and outcomes displayed in table format. The authors consider a meta-analysis inappropriate of the T$_2$ relaxation values, due to heterogeneity in data acquisition, voxel location, gender, and age; however, we acknowledge the importance and utility of visualizing the outcomes in comparison to an overall average per magnetic field strength and metabolite by forest plots. Overall, NAA has shown a strong separation between GM, WM and mixed tissue at 1.5T and 3T, while Cr displayed differences between GM and WM at 3T. Changes were observed between Cho GM and mixed tissue at 1.5T. Other metabolites at different magnetic field strengths were not considered for evaluation due to limited research. However, inferences from this review data have to be considered with caution.

Can the differences between GM and WM in NAA and Cr T$_2$ values be attributed to their compartmentation and hence their local environment? In literature NAA is often regarded as a marker of neuronal state as a result of its predominant location in neurons, however its function is not yet entirely understood (Ross & Bluml, 2001). In brief, NAA is synthesized in neuronal cell bodies and catabolized in glial cells (oligodendrocytes) in GM (Rae, 2014). There are mixed reports of differences in NAA concentration levels in healthy adults in GM and WM, however it is proposed that NAA is present in axons in WM (Kreis, 1997; Ross & Bluml, 2001). Research into Cr indicates it is located in both astrocytes and neurons, with mixed reports about Cr's concentration levels in each location (Mountford et al., 2012; Rae, 2014). Notably, astrocytes can be divided into two groups either protoplasmic or fibrous, depending on whether they are located in GM or WM, respectively (Sofroniew and Vinter, 2010). By extension, the shorter NAA and tCr T$_2$ values in GM may imply less free movement due to more structure in cell bodies of neurons and protoplasmic astrocytes compared to movement in axons and fibrous astrocytes in WM (Frahm et al., 1989; Kreis et la., 1993). Presumptively, limited movement in cell bodies may be due to NAA and Cr being bound up with mitochondrial membrane and/or proteins, while the increased T$_2$ values in WM

may indicate more mobility due to less compartmentation (Cady et al., 1996). Although NAA T$_2$ differences were observed between WM and GM as well as WM and mixed tissues at 1.5T and 3T this does not apply to Cr, which only showed a difference for GM and WM. In contrast, Cho is present in neurons and astrocytes the same as Cr (Rae, 2014), nonetheless Fig. 6 and Fig. 7 illustrate the lack of difference in T$_2$ between GM and WM implying perhaps that the local environment of Cho differs little between these two cellular locations. The difference in Cho T$_2$ values between GM and mixed tissues seems to be affected by the T$_2$ value reported by Brooks et al. (2001) of 556ms with a standard deviation of 214ms. The authors recruited a total of 50 male participants of which 10 participants were allocated per decade (20 and 70 years). It is possible that this broad range affected the large standard deviation. However, Brooks et al. (2001) observed no significant changes in T$_2$ relaxation with respect to age. As such, the findings should be considered with caution.

3.5.2. Methodological challenges

The second aim of this review was to identify and discuss possible obstacles associated with T$_2$ measurements in research. Here we will address the following methodological challenges: TE, J-coupling, and exponential fit.

The reviewed studies presented a mix of TE ranges from 1ms to 1500ms for their T$_2$ measurement. The selection of a suitable TE in magnetic resonance experiments is dependent on the information being sought; the TE selected can be used to manipulate the signal dependent on either the desired tissue contrast (MRI and fMRI studies) or desired metabolites (MRS studies) (Brief et al., 2005; Huettel et al., 2009). TE is a time interval, which represents the time between the application of an excitation pulse and the acquisition of the signal at its highest point (Huettel et al., 2009). Research literature often distinguishes between short and long TE with each having strengths and weaknesses. However, there is no definite cut-off point for what constitutes a short or long TE. A short TE minimizes T$_2$ loss as transverse magnetization is not lost yet and exposes more metabolites with shorter decay compared to longer TEs (Brief et al., 2005; Soher et al., 2005). The weaknesses of short TE MRS acquisitions are macromolecule and lipid overlaps as well as complicated spectral patterns due to J-coupling effects, which can lead to difficulties in analysing spectroscopy data (Brief et al., 2005; Soher et al., 2005). Long TE avoids some of these difficulties as only those metabolites with long T$_2$ relaxation are observed (Soher et al., 2005) and signal modulation due to J-coupling effects may lead to simplified spectra (Mullins et al., 2008; Schubert et al., 2004). Moreover, longer TE may experience signal loss in excess of T$_2$

decay if eddy currents are not appropriately corrected for (Kreis, 2004). As noted earlier T$_2$ relaxation values are an approximate calculation and during their quantification uncertainties may be introduced from other factors (movement, diffusion, local field in-homogeneities) resulting in under- or overestimation of T$_2$ values (Brief et al., 2005; Henning et al., 1993). Rutgers and van der Grond (2002) proposed that studies attempting measure T$_2$ relaxation it is preferable to include longer TEs, preferably over 272ms, to capture more signal decay from NAA and Cr. Notably, the authors cautioned that their T$_2$ values may be underestimated due to an absence of accounting for macromolecular decay. Further support for this suggestion comes from Brief et al. (2005). The authors noted that the use of a maximum TE of 272ms in an experimental study might result in overestimation of metabolite T$_2$ values. However, the errors may also occur if the TE range is too large due to reduced signal-to-noise ratio (SNR) at the longest TEs. Another essential point to consider is the number of TEs used. Mason et al. (1995) reported that 2 TEs would be sufficient to estimate T$_2$ values taking into account explicit calculation of uncertainties, but it may also be argued that more points on the decay curve will give better estimates.

There is limited research on T$_2$ relaxation times of coupled spin systems (such as Glu, GABA or MI), as they are not only modulated by T$_2$ relaxation but also J-coupling (Choi et al., 2006; Deelchand et al., 2012; Edden et al., 2012; Intrapiromkul et al., 2013). Furthermore, in general, the longer the TE the less signal is available, however, with a shorter TE the contribution of macromolecules (MM) to signal intensity measures increases (Marjańska et al., 2012; Schubert et al., 2004). Some researchers have proposed simulation of spectral patterns at each TE to reflect the signal changes due to J-coupling will allow the implementation of a model to which in vivo data can be fit (Choi et al., 2006; Edden et al., 2012; Intrapiromkul et al., 2013; Marjańska et al., 2012).

In addition to the choice and number of TEs used, the method for calculating the decay constant also needs to be considered. The literature reviewed revealed, where identifiable, the mono-exponential fit of signal decay as the most frequently used method for T$_2$ estimation. The mono-exponential fit is used due to its relative simplicity. Frahm et al. (1989) reported that a mono-exponential fit would be sufficient for fitting Cr T$_2$ relaxation values. However, this two-component resonance signal derives from creatine and phosphocreatine (PCr), which may imply that both have different T$_2$ relaxation times. Ke et al. (2002) reported PCr T$_2$ relaxation time to be shorter in pure water compared to Cr T$_2$ relaxation as well as suggesting that a bi-exponential decay model would be more suitable to fit tCr. Whitall et al. (1999), suggests that fitting WM and GM to mono-exponential fits results in introducing errors

that could be avoided when using a multi-exponential analysis. However, NAA seems not to experience the same bi-component decay model but rather a mono-exponential decay. Further research is required to further investigate the decay curves of metabolites such as Glu, Gln, MI, and GABA, however these are further complicated by J-modulation. Despite the possibilities of more complicated decay curves, a monoexponential fit is still likely to be of use in providing estimates of T$_2$ decay.

3.5.3. Consensus T$_2$ values

The final aim of this review was, where possible, to present consensus T$_2$ values for researched metabolites in regions of GM, WM, and mixed tissue content. The findings for NAA show a clear separation between WM and GM T$_2$ relaxation mean values at 1.5T and 3T. Additionally, the WM T$_2$ relaxation mean value for NAA was significantly different from mixed tissue at 1.5T and 3T. Therefore, when performing relaxation correction for NAA estimates using 1H-MRS we recommend the use of the mean T$_2$ relaxation values reported according to tissue content of the voxel of interest: at 1.5 T – 415ms for WM, 335ms for GM, and 343ms for mixed tissue; and at 3T 346ms for WM 268ms for GM and 249ms for mixed tissue. The results for Cr showed a significant difference between WM and GM T$_2$ relaxation mean values at 3T. As such, we advise to use T$_2$ relaxation mean values based on voxel content: at 1.5 T – 206ms for WM, 201ms for GM, and 215ms for mixed tissue; and at 3T 175ms for WM 156ms for GM and 163ms for mixed tissue. The outcome for Cho T$_2$ relaxation mean values at 1.5T suggests a significant difference between GM and mixed tissue. T$_2$ relaxation mean values should be used accordingly with tissue content: at 1.5 T – 325ms for WM, 377ms for GM, and 324ms for mixed tissue. The findings for Cho at 3T suggest no differences between WM, GM, and mixed tissue T$_2$ relaxation mean values. Therefore, we suggest the use of an overall T$_2$ mean value of 236ms at 3T. These recommendations of metabolite T$_2$ relaxation mean values should be considered with care due to variations in studies such as scanner as well as acquisition or processing parameters, but do represent the best consensus currently available.

3.5.4. Metabolite quantification in early life and old age

As noted earlier, T$_2$ relaxation is pivotal for the quantification of metabolite concentrations, as it contributes to the peak area (Barker et al., 2001). The review findings suggest a clear difference of NAA T$_2$ relaxations between GM and WM. If differences in T$_2$ measures are not considered this may cause incorrect assumptions implying that NAA concentrations are

lower in GM then WM, if not properly accounted for. This can be a concern when conducting research into ageing and disease. Potential T$_2$ changes may reflect concentration changes and result in inaccurate outcomes concerning ageing and disease progression.

Research in early life studies has investigated NAA, Cr, and Cho T$_2$ relaxation times at 1.5T and 2.4T (Cady et al., 1996; Kreis, Ernst & Ross, 1993; Kugel et al., 2003; Toft, Leth et al., 1994; Toft, Christiansen et al., 1993). The infants were between gestational ages of 30 - 930 weeks (Toft, Christiansen et al., 1993). Kreis, Ernst and Ross (1993) reported of a trend in 30% increase in NAA T$_2$ relaxation in infants compared to adults. Additionally, the authors observed a trend in 10% increase in Cr, Cho, and MI in infants compared to adults. Toft, Christiansen et al. (1993) pointed out that using four TEs, NAA T$_2$ relaxation was significantly longer compared to using only three TEs. Toft, Leth et al. (1994) suggested an inverse correlation between PCr, Cho, and NAA with gestational age. Whereas, Cady et al. (1996) reported of a positive correlation between thalamic NAA and gestational age. However, the authors did also find an inverse correlation between thalamic Cho and gestational age.

Research into age-related T$_2$ relaxation differences has investigated the principal metabolites NAA, Cr, and Cho at 1.5T, 3T, and 4T (Brooks et al., 2001; Christiansen et al., 1993; Kreis et al., 2005; Longo et al., 1995; Kirov et al., 2008; Marjańska et al., 2013). At 1.5T three studies reported of no significant differences in T$_2$ relaxation times in mixed and mostly grey matter with age (Brooks et al., 2001; Christiansen et al., 1993; Longo et al., 1995), however, one study suggested NAA T$_2$ relaxation increases in WM across age (Kreis et al., 2005). Kirov et al. (2008), in a multi-voxel study, reported shorter NAA, Cr, and Cho T$_2$ relaxation times in GM compared to WM at 3T with age. A further study, conducted at 4T, suggested shorter T$_2$ relaxation times for NAA, Cr, and Cho with age in the occipital lobe. In addition, Marjańska et al. (2012) reported significant differences between tissue water T$_2$ values across age in the occipital lobe. Brain water is a validated internal quantification reference for estimation of metabolite concentration (Gasparovic et al., 2006). Therefore, tissue water T$_2$ relaxation also has to be considered. Christiansen, Toft et al., (1993) used brain water as an internal reference and reported similar metabolite concentration levels as previous research using an external reference. The authors found significantly lower brain water T$_2$ relaxation values in the basal ganglia compared to occipital, temporal, and frontal brain regions in younger and older participants. Water in GM is suggested to have longer relaxation compared to WM (Christiansen, Henriksen et al., 1993). As such, T$_2$ relaxation times of the metabolites NAA, Cr, and Cho along with water appear to be region and age dependent.

Overall, the literature provides mixed findings in regard to metabolite T$_2$ changes across lifespan. Certainly, the differences in findings can be attributed to one or more factors such as field strength, acquisition and processing parameters as well as research groups. In light of this further research conducted into age-related T$_2$ changes are warranted, investigating broader age ranges with increased sample sizes.

3.6 Conclusion

This review provides a collection of studies investigating T$_2$ relaxation times in the human brain. Consensus T$_2$ relaxation values are suggested for NAA, Cr, and Cho at 1.5T and 3T. There are factors such as TE, J-coupling, and exponential fit that can introduce uncertainties into T$_2$ relaxation quantification, which, in return would effect metabolite concentration. Equally important, researchers have to be aware of other factors that can have an effect on T$_2$ relaxation such as compartmentation, ions, cellular structure, post-processing, etc. (Frahm et al., 1989). However, T$_2$ relaxation quantification is not a straightforward process and further research is still required to better establish T$_2$ relaxation times for other metabolites such as Glu, GABA or MI.

Chapter 4

Posterior Cingulate Cortex T$_2$ Relaxation Times and Concentration Levels of Proton Metabolites in Ageing Brain at 3 Tesla

4.1. Abstract

The study's aim was to investigate possible age-related effects on measures of neuro-metabolite transverse relaxation (T$_2$) times within the posterior cingulate cortex (PCC), an important area in ageing and dementia research, and associated effects this has on concentration estimation. Proton magnetic resonance spectroscopy was utilized to acquire NAA, choline, creatine, glutamate, and myo-Inositol T$_2$ relaxation times and concentration measures from 13 healthy young and 11 typically ageing older adults. As T$_2$ decay is affected by contributions of internal and external factors such as diffusion, the term T$_2^{\dagger}$ will be used instead to describe apparent decay. Measures revealed no statistically significant differences of T$_2^{\dagger}$ for creatine, myo-Inositol and glutamate between older and younger adults, however a decrease in T$_2^{\dagger}$ for NAA and a trend for choline were seen ($p = 0.007$, and $p = 0.027$, respectively). When using cohort estimated and literature T$_2$ values for relaxation correction, previously reported age-related differences in metabolite concentrations were no longer found, although a trend in glutamate ($p = 0.037$) decrease was present. NAA T$_2^{\dagger}$ relaxation exhibits age-related decline, suggesting micro-environmental changes within PCC's neurons. When using age appropriate T$_2^{\dagger}$ values for relaxation correction in metabolite concentration estimation, previous age-related declines in NAA are no longer seen. These findings reinforce the importance of T$_2$ relaxation measurements and the use of appropriate relaxation corrections in MRS quantification.

Keywords: ageing, T2, transverse relaxation, MRS, PCC, posterior cingulate

4.2. Introduction

Research into dementia has shown a growing interest in the posterior cingulate cortex (PCC) due to its decreasing volume in Alzheimer's disease (AD) compared to healthy age-matched controls (Choo et al. 2010). The PCC, situated in the limbic lobe, is distinguished by increased metabolism at rest and considered to hold a primary role in the default mode network (DMN) (Greicius, Krasnow, Reiss, & Menon, 2003; Rajmohan & Mohandas, 2007). Abnormalities such as hypometabolism, amyloid deposition, intracellular neurofibrillary tangles and functional irregularities in the PCC have been linked with mild cognitive impairment (MCI) and Alzheimer's disease (AD) (Buckner et al., 2005; Nishi et al., 2010; Zhou et al., 2008). In addition, research into ageing has proposed similar structural and functional changes may occur, to a lesser extent (Aizenstein et al., 2008; Takahashi, Ishii, Kakigi, & Yokoyama, 2011). Advancements in imaging as well as attempts to find a biomarker for disease onset, have further facilitated research investigating changes at the molecular level in the PCC in ageing and neurodegenerative processes associated with dementia (Chiu et al., 2014; Reyngoudt et al., 2012; Wang, Zhao, Yu, Zhou, & Li, 2009).

Proton magnetic resonance spectroscopy (^{1}H-MRS) is utilized to non-invasively measure neuro-metabolite concentration levels in vivo (Kreis, 1997). The principal ^{1}H-MRS metabolites investigated in ageing and dementia research are N-Acetyl Aspartate (NAA), choline (Cho, the sum of choline, phosphocholine and glycero-phosphocholine), creatine (Cr, the sum of creatine and phosphocreatine), and myo-Inositol (MI), although recently focus has shifted to include the neurotransmitters glutamate (Glu) and γ-aminobutyric acid (GABA) (Gao et al., 2013; Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013; Haga, Khor, Farrall, & Wardlaw, 2009; Reyngoudt et al., 2012). The most consistent findings from AD studies propose a decrease in NAA concentration levels as a ratio to either Cr, Cho, MI, or water, compared to a healthy age-matched population in the PCC (Garcia Santos et al., 2008; Watanabe, Shiino, & Akiguchi, 2010). Some research has reported increases of MI/Cr and MI/NAA in AD patients compared to healthy controls, while mixed findings have been reported for Cho (Silveira de Souza, de Oliveira-Souza, Moll, Tovar-Moll, Andreiuolo, & Bottino, 2011). There has been very little ^{1}H-MRS research investigating age-related differences in the PCC compared to dementia research (Chiu et al., 2014; Reyngoudt et al., 2012). One of the few studies reports increased levels of absolute concentrations for Cho, Cr and NAA, while no changes were observed in MI with age (Chiu et al., 2014). A second study found increased concentration levels in tCr, tCr/H$_2$O, MI, MI/tCr, and MI/H$_2$O with age. However, Cho/tCr was reported to decline with age while Cho/H$_2$O and NAA showed no

significant differences across age (Reyngoudt et al., 2012). Both studies reported correcting for metabolite and water transverse relaxations (T$_2$), however only one study reported water T$_2$ relaxation values (Reyngoudt et al., 2012). The mixed findings for both studies may reflect potential variations in metabolite T$_2$ relaxation times as well as technical and methodological differences.

To estimate accurate metabolite concentrations correct measures of T$_2$ relaxation times should be acquired and used (Kreis, Ernst, & Ross, 1993). Without correct measures, concentration estimates may contain errors, which in turn could lead to wrongful interpretations of age and disease related concentration differences. While previous research has investigated age-related effects of metabolite T$_2$ relaxation times at 1.5T (Brooks et al., 2001; Christiansen, Toft, Larsson, Stubgaard, & Henriksen, 1993; Kreis, Slotboom, Hofmann, & Boesch, 2005; Longo, Bampo, Vidimari, Magnaldi, & Giorgini, 1995), 3T (Kirov et al., 2008) and 4T (Marjańska, Emir, Deelchand, & Terpstra, 2013), the outputs from these studies present mixed results in regard to NAA, Cho and Cr T$_2$ relaxation times across age. Research conducted at 1.5T has proposed no significant differences in age-related T$_2$ relaxation times aside from the study by Kreis et al. (2005) reporting NAA T$_2$ increases with age. In contrast a multi-voxel study performed at 3T (Kirov et al., 2008) indicated shorter NAA, Cr and Cho T$_2$ relaxation times across age, which was supported by a further study at 4T representing similar results (Marjańska et al., 2013). Presently there is no data on possible changes in T$_2$ relaxation for Glu with age.

The primary aim of this study therefore was to determine proton metabolite transverse relaxation times for NAA, Cho, Cr, MI and Glu in both, young and older adult cohort. A secondary aim was to use the same data to investigate corrected metabolite concentration levels in the posterior cingulate cortex (PCC). This is especially important considering the growing interest of the PCC's role in MCI and AD research (Choo et al. 2010).

4.3. Methods

4.3.1. Participants

A total of 13 healthy younger (8 females, aged 18 – 41, $M \pm SD$ = 24.46 ± 6.3 years) and 11 typical ageing older (8 females, aged 59 – 80, $M \pm SD$ = 67.55 ± 6.2 years) participants were recruited from the student and local community. All participants had no known history of neurological conditions, however some of the older participants took medication for

hypertension (n = 5), thyroid dysfunction (n = 3) and cholesterol (n = 3), potential effects are addressed in the discussion. Exclusion criteria were based on self-reported history of psychiatric and neurological disorders as well as failing to meet the MRI safety screening form exclusion criteria. Bangor University School of Psychology Ethics and Research Committee approved all protocols (2012-6522-A13630) and written informed consent was obtained prior to scanning. Participants received monetary compensation for their time.

4.3.2. Data Acquisition and Processing

All scans were acquired with a SENSitivity-Encoded (SENSE) 32-channel head coli on a 3T Philips Achieva MR scanner (Philips Health Care, Eindhoven, Netherlands). T$_1$-weighted images (MP-RAGE; FOV = 230 x 230 x 140mm; TE = 4.4ms; TR = 24ms; slice thickness = 0.7mm; flip angle = 8°) were acquired for localization and referencing of MRS voxel and followed by five spectroscopy scans applying Point RESolved Spectroscopy (PRESS; Bottomley, 1987). Spectra were acquired by placing a single voxel of 20 x 20 x 20 mm^3 in the PCC, which contained mixed white (WM) and grey matter (GM) tissue. CHESS water suppressed spectra were acquired at TR = 2000ms and varying TE of 40, 80, 105, 256, and 400ms with 2048 data points. To compensate for signal-to-noise ratio (SNR) loss at TE 256 and 400ms a total of 256 averages was acquired, compare to 128 averages at TE 40, 80, and 105ms.

Each ^{1}H-MR spectrum was analysed in the time-domain using TARQUIN 4.3.8 (Wilson, Reynolds, Kauppinen, Arvanitis, & Peet, 2011) software. Preprocessing of the acquired signals consisted of automatic zero-order phasing and referencing, which was followed by removal of residual water resonances at a cut off of 45 Hz with the Hankel Singular Value Decomposition (HSVD) method. To lessen baseline interference the first 10 and last 1024 points were truncated. Fitting was performed using the 1H brain full internal basis set supplied in TARQUIN, which is comprised of the following metabolites: alanine (Ala), aspartate (Asp), creatine (Cr), gamma-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycerophorylcholine (GPC), glycine (Gly), guanidinoacetate (Gua), myo-inositol (MI), lactate (Lac), N-acetyl-aspartate (NAA), N-acetyl-aspartylglutamate (NAAG), phosphorylcholine (PCh), phosphocreatine (PCr), scyllo-inositol (s-Ins), taurine (Tau), and incorporated models for the macromolecular (MM) and lipid components. Cr and PCr values were combined for Cr estimation as was Cho, GPC, and PCh values for tCho estimation. Results of the fit for each metabolite were scaled to an unsuppressed water scan collected at TE = 40ms with 16 averages from the same region. In doing so acquisitions were

also corrected for the differing number of averages by a scaling factor incorporated in TARQUIN. This ensures the acquisitions with 256 averages (TE = 256 and 400ms) are scaled appropriately (e.g. by 0.5) to account for the twofold increase in signal due to the extra averages. While scaling the processed signal, water concentration was set to 55.55 mol/l within TARQUIN. In addition, the water attenuation factor was set to 1. This factor is usually set to 0.7 by default and is used to account for relaxation effects on the water signal, however this is based on the assumption of a white matter voxel, at 1.5 T and a TE of 30 ms. As our data were collected at 3T in mixed tissue, we corrected for water content and possible water relaxation effects in a separate step after fitting. Line-broadening of 2 Hz was applied to the acquisitions during post-processing for visual inspection only and had no impact on spectral fitting estimations (Figure 1).
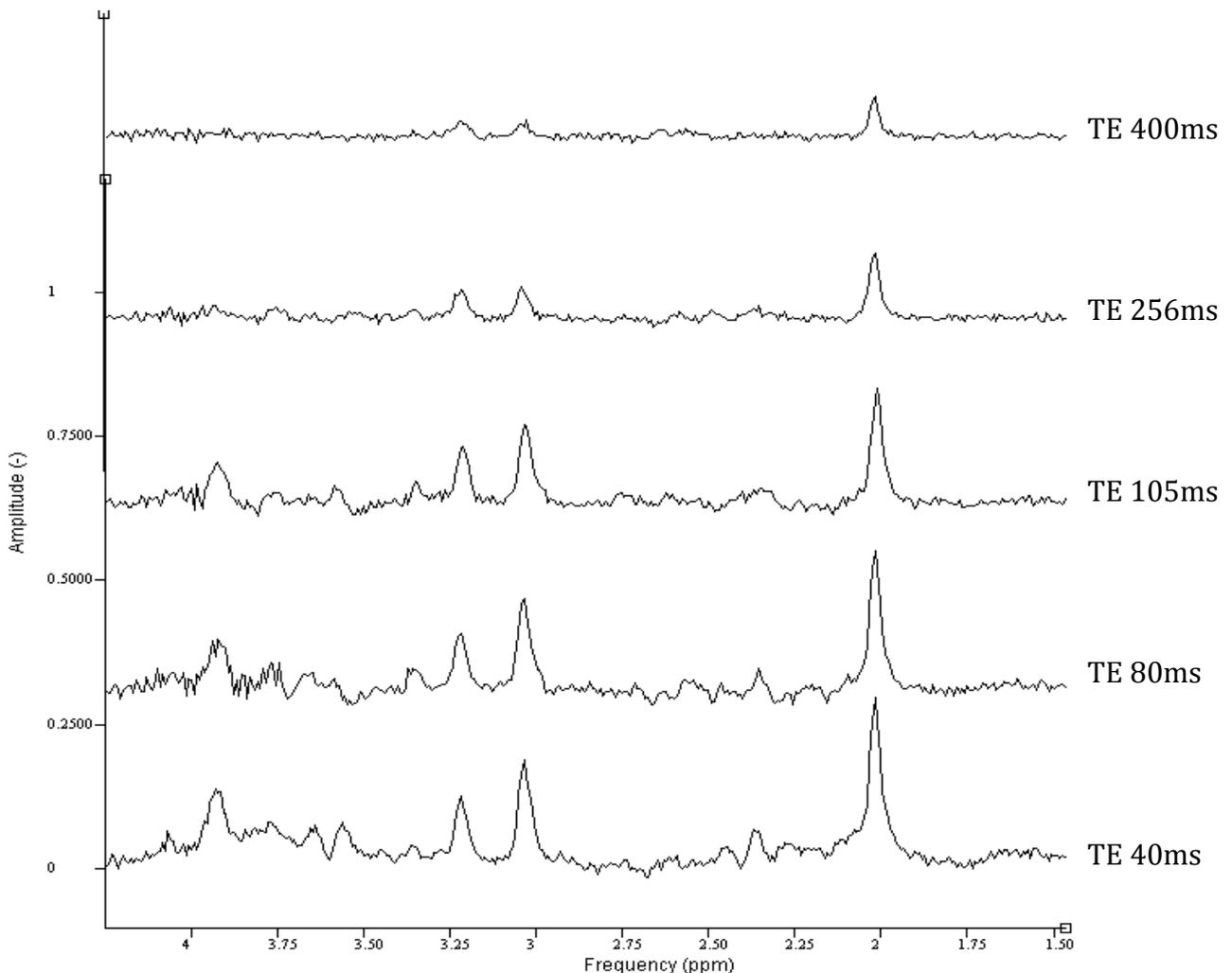
Figure 1. Example of PRESS spectra obtained with TR = 2000ms and TE$_{1-5}$ = 40/ 80/ 105/ 256/ 400ms. TE is shown on the right hand side of each spectra. However, the spectra for 256ms and 400ms have not been corrected for double acquisition in this figure.

A mono-exponential log-linear fit to the signal decay over echo time enabled the T_2 relaxation time for each metabolite to be estimated. A partial volume correction code was used to establish GM, WM, and CSF percentages of the voxel of interest (VOI) to correct for partial volume and relaxation effects in order to get corrected metabolite concentration levels from the TE 40ms data (for equation please see Gasparovic et al., 2006). The present study's metabolite T_2 values and literature values were used to correct for relaxation effects in these estimated concentrations, while metabolite T_1, and water T_1 and T_2 values were based on literature values (Table 1). It should be acknowledged measurement of transverse relaxation in vivo will produce an apparent T_2 relaxation rate, given that signal decay in a spin echo experiment is also affected by diffusion, flow, movement and j-modulation effects as well as the choice of echo times, number of echo times and spin echo experiment. By empirically measuring the decay of the signal for metabolites a researcher should capture these additional effects, in a single measure of apparent decay. Apparent T_2 decay is usually represented as T_2^*, however this term is more commonly used to refer to the effects of local field inhomogeneity and the BOLD (blood-oxygenation-level dependent) effect. As such we will use the term $T_2^{\dagger}$ for our results.

	NNA	Cho	Cr	Glu	MI	Water (GM)	Water (WM)	CSF
T_1	1403	1182	1320	1220	1102	1488	781	4000
T_2	247	254	160	169	200	71	58	200

Table 1. Presented are averaged literature T_1 values for metabolites and water as well as water T_2 values in ms. While metabolite T_2 values are an average of literature and this study's T_2 values.

4.3.3. Statistical Methods

All statistical tests were performed using JASP (Love et al., 2015). Independent t-tests (2-tailed, standard and Bayesian) were used to compare $T_2^{\dagger}$ relaxation times and metabolite concentration levels between younger and older adults using the results of the TE = 40ms PRESS acquisition. As 5 metabolites were investigated Bonferroni adjustment was applied to correct for multi-comparison with a P value of 0.01 being considered significant in the standard tests. For Bayesian factor analysis we assume a BF_{+0} between 0 - 1 as not

significant, 1 - 3 as anecdotal, 3 - 10 as moderate, 10 - 30 above as strong, and above 30 as very strong support of the hypothesis. As a secondary analysis of T$_2$† effects, and a measure of scan quality we also compared the line width (full width at half maximum – FWHM) for water and NAA between the two cohorts.

4.4. Results

Figure 2 shows a sample of an individual exponential T$_2$ fit for NAA for a younger adult. Figure 3 displays T$_1$ – weighted images with VOI in one older (a) and one younger (b) participant along with the affiliated spectrum. T$_2$† relaxation mean values for NAA, Cho, Cr, Glu and MI are summarised in Table 2. The findings revealed shorter T$_2$† mean values for NAA, Cho, and MI by 9.4%, 9.1%, and 7.8% in older compared to younger participants, however only the NAA results survive correction for multiple comparisons ($p = 0.007$, Cohen's d = 1.26), while the Cho changes remain at the level of a trend after correction for multiple comparisons ($p = 0.027$, Cohen's d = 0.97). No significant differences were observed in the T$_2$† values for Cr, Glu and MI. Bayesian analysis of the NAA T$_2$† rates also showed strong support for the hypothesis that T$_2$† values are lower in the older participants (BF$_{+0}$ = 13.16) with moderate support for a reduction in the T$_2$† of Cho with age (BF$_{+0}$ = 4.98).

$$\log(\text{signal}) = -TE/250 + 3.09$$
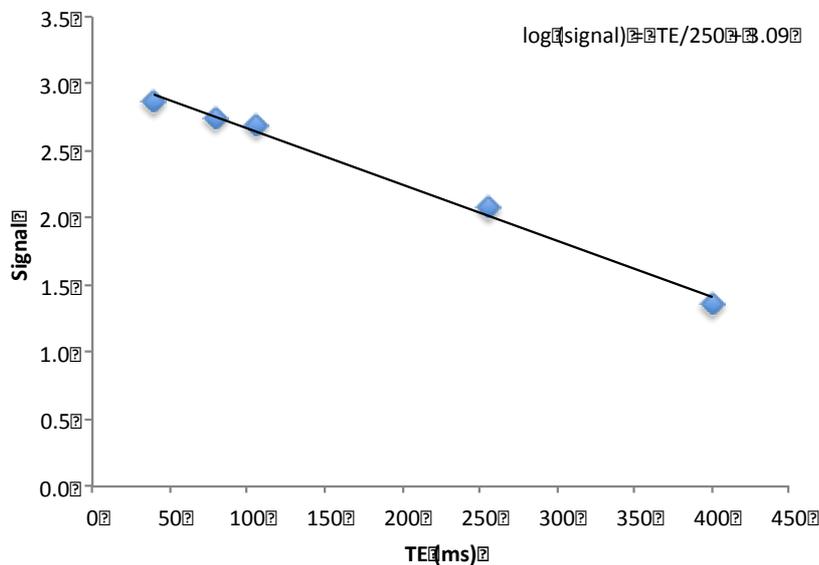
Figure 2. Example of log plot to determine NAA T$_2$ relaxation time (-1/slope).
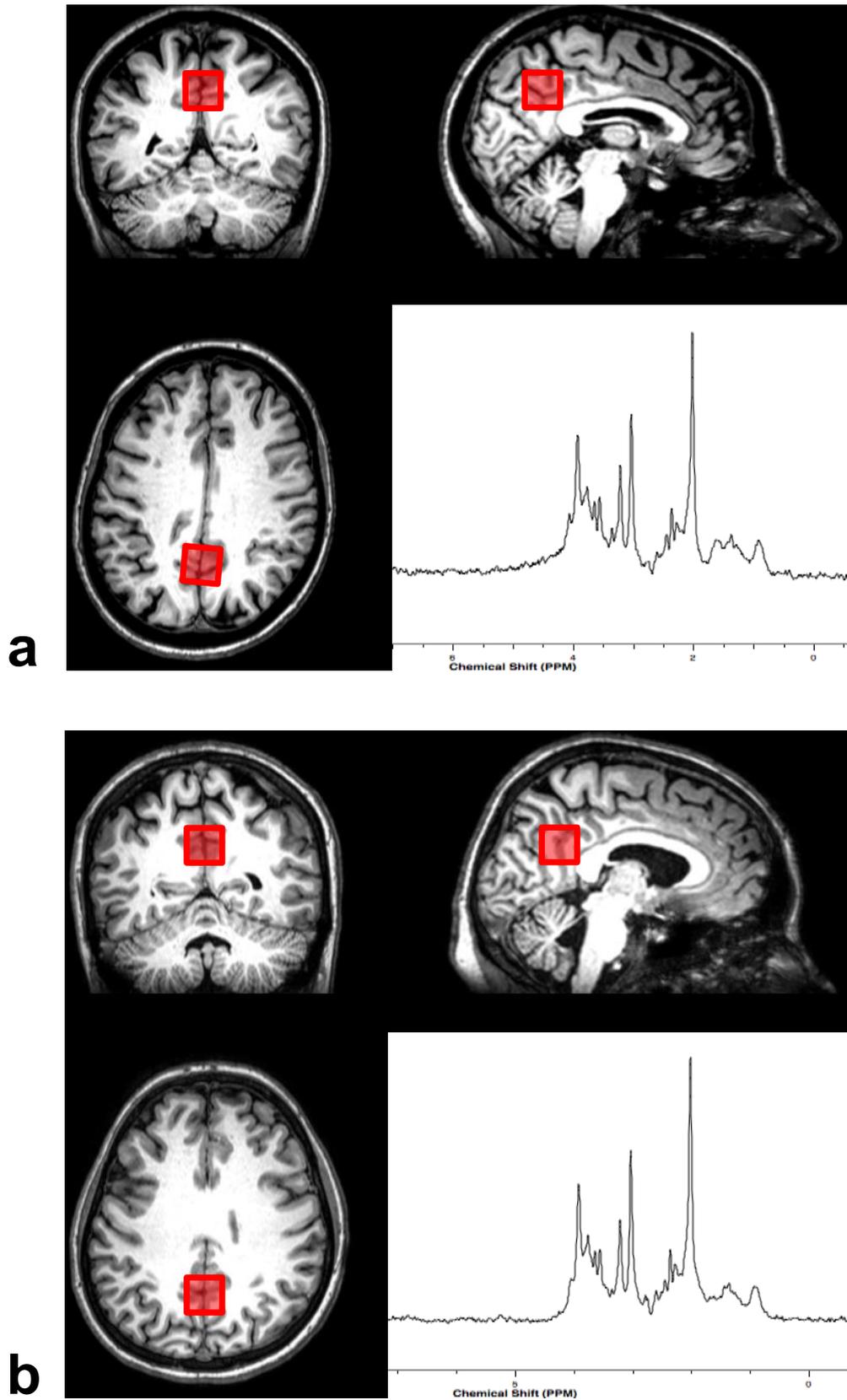
Figure 3. Example of voxel placement in older (a) and younger (b) participants along with corresponding spectrum acquired at TE 40ms.

	NNA	SD	Cho	SD	Cr	SD	Glu	SD	MI	SD
Younger	254	20	275	25	150	7	125	16	191	50
Older	230	18	250	29	145	9	127	17	176	50

Table 2. Summarized are mean $T_2^{\dagger}$ relaxation times and standard deviation (SD) by younger and older participant in ms at 3T.

PCC's mean metabolite concentration levels for NAA, Cr, Cho, Glu and MI, corrected by merging both literature assumed T_2 and currently reported mean $T_2^{\dagger}$, are outlined by younger and older participants in Table 3. No significant age-related differences were observed in concentration levels of NAA, Cho, Cr, and MI. Glu was observed to decrease with age, but this was only at a trend level after correction for multiple comparisons ($p < 0.037$), the Bayesian analysis similarly shows only moderate support for a reduction in Glu in the older cohort ($BF_{+0} = 4.5$).

	NNA	SD	Cho	SD	Cr	SD	Glu	SD	MI	SD
Younger	12.4	0.7	1.7	0.2	11.9	0.5	19.5	2.5	6.7	0.9
Older	12.1	1.3	1.5	0.2	11.6	0.7	17.3	2.3	6.4	0.8

Table 3. Displayed are mean metabolite concentration levels millimolar (mM) with SD of younger and older participants in the PCC.

Examination of the data used for partial volume effects showed a significant difference in WM ($p = .003$) and CSF ($p = .01$) but not GM ($p = .321$) between the two cohorts. Older participants had higher CSF (+4%) and lower WM (-5%) but an insignificant increase in GM (1%).

TARQUIN's fit quality (Q) mean values for younger and older participant were 1.42 and 1.47, respectively. A value between 1 and 2 is considered a good fit.

TARQUIN also provides a measure of the full-width-at-half-maximum (FWHM) for the NAA peak and water. Comparison of this produced mean values of 4.15 Hz and 5.13 Hz for younger and older participants, respectively. As increased line width is also related to a decrease in T$_2$ it is of interest to note that the NAA FWHM for older participants is significantly higher (p = .001, Cohen's d = -1.89, BF$_{+0}$ < 100) than found in younger participants. FWHM for water was found to be the same for both cohorts (Younger = 6.4 Hz, Older = 6.3 Hz).

4.5. Discussion

4.5.1. Transverse relaxation across age

The first aim of this study was to determine metabolite T$_2$† relaxation times in the PCC across age. Our findings indicated shorter mean T$_2$† relaxation times for NAA, Cho, and MI in older compared to younger participants, however of these differences only the NAA difference was considered statistically significant when correcting for multiple comparisons. This reduction in NAA T$_2$† is not entirely surprising and matches with previously reported age-related reductions in NAA relaxation times in various grey and white matter brain areas, including occipital cortex, at 3T and 4T (Kirov et al., 2008; Marjańska, Emir, Deelchand, & Terpstra, 2013), but disagrees with a previous finding at 1.5 T, which suggests T$_2$ increases with age (Kreis, Slotboom, Hofmann, & Boesch, 2005). This reduction in the T$_2$† was further backed up by an observed increase in the line width for NAA, while the water line width was unchanged. It is possible that tissue composition differences between the young and old cohort could have also affected T$_2$† measures. We found a 5% reduction in WM, and a compensatory 4% increase in CSF in the older cohort with no significant change in GM (+1%). While T$_2$† for NAA is reported to be shorter in GM then WM, and a reduction in WM would lead to a reduction in T$_2$†, this reduction would have been greater if there was a concomitant increase in GM. However, mean GM was only 1% higher, and we are not sure the effect would be as marked as seen. Indeed taking a literature average WM T$_2$† for NAA as 345ms, and an average GM T$_2$† of 268ms, we would expect the T$_2$† for our younger participants to be around 255ms based on the tissue content (actual measure was 254ms), while for the older cohort we would expect a mean T$_2$† of ~ 241ms based on tissue content, which while reduced is not as reduced as the mean of 230ms we report. This, combined with the line width changes, suggests the reduction in T$_2$† we report is more then just a result of tissue content changes.

A reduction in the measured T$_2$[†] for NAA is informative as it suggests age-related changes in the micro-environment that NAA inhabits. As NAA is restricted to the neurons, we might infer this means a change within the neuronal cytosol. This finding also has implications for studies that report a reduction in NAA with ageing. A reduction in T$_2$[†] will also lead to less signal, which could be mistaken for a reduction in the metabolite if appropriate corrections for relaxation effects are not applied especially, if a long TE is used.

We did not detect a similar reduction in T$_2$[†] for Glu, indeed Glu T$_2$[†] values are the same for both cohorts, suggesting that most MRS visible Glu is localised to a different environment from NAA, and undergoes different interactions with it's surroundings. It is important to be cautious when determining MI and Glu T$_2$[†] relaxation values as both are scalar coupled metabolites giving rise to multiple peaks which are affected by J-coupling, overlap with other metabolites, and are contaminated by MM (Choi et al., 2006; De Graaf & Rothman, 2001; Ganji et al., 2012; Puts & Edden, 2012; Schubert, Gallinat, Seifert, & Rinneberg, 2004). J-coupling can negatively impact T$_2$[†] measures by adding an additional reduction in the overall peak amplitude with echo time, while peak overlap and increased macromolecular contamination may reduce the reliability of the fit. Use of simulated peak models that take into account the effects of J-coupling, and fitting the entire spectral pattern for each metabolite, as opposed to one exemplar peak, may reduce the impact of J-coupling on signal reduction with increasing TE, however it does introduce another complication. Use of the entire spectrum for fitting assumes that T$_2$[†] relaxation is the same for all protons on the molecule in question, this may not be a safe assumption in the complicated milieu of brain tissue, and as such any T$_2$[†] reached by such a method is an "average T$_2$". In addition, the choice of echo times has been shown to have an effect on the T$_2$[†] measures obtained and it is possible that use of a different range of TE's may have produced a difference in T$_2$[†] values being reported, however our range was chosen based on both previous reports, and the expected T$_2$[†] values for our metabolites, and was a compromise chosen to give the best chance at reliable results for all metabolites.

We mention these as caveats for both our T$_2$[†] data and previous results, and suggest careful consideration of the methods used always to be included in any comparisons or judgements on validity of results. Despite these caveats, our results for most metabolites in the younger cohort closely match those previously reported (Ganji et al., 2012; Kirov et al., 2008; Träber, Block, Lamerichs, Gieseke, & Schild, 2004; Zaaraoui et al., 2007)(and Chapter 3) with only Glu showing a difference in T$_2$[†], which could be a result of the complications in measuring T$_2$[†] for this metabolite already mentioned, or as a result of slightly differing measurement

methods. For instance, Schubert et al. (2004) mention their T$_2$ values for Glu are only effective T$_2$ results, as they are referenced to phantom data acquired at the same echo times, while Ganji et al. (2012) used different echo times, particularly a longer first TE of 54ms. In addition, these measures where both made in differing regions and in a much smaller cohort then this study (n = 3, and n = 5 respectively compared to n = 24 (total young + old). There are also differences in the fitting software and how MM are accounted for within the models used. While TARQUIN does include models of the MM in the basis set used for fitting, it may perform differently to LC-Model and slightly underestimate the MM contribution therefore overestimating the Glu peak at shorter TE's, which in our results may lead to an overestimation of the rate of signal decay. To address this potential complication we re-analysed the data using the 80ms TE (with minimal MM contamination) as our first TE. While the average T$_2$† estimated in this fashion did increase slightly to 147ms, this is still much less then the previously reported value for Glu of 181ms. The differences in T$_2$ measures between this report and previous data highlights that more work needs to be done to confirm the T$_2$ for Glu and to reach a consensus, although it is reassuring that our Glu measures were consistent across both cohorts, with higher n then previously reported, improving the reliability of these results.

4.5.2. Metabolite concentrations across age

A secondary aim of the current study was to investigate metabolite concentration levels in the PCC across age using age appropriate measures of T$_2$†. As with measures of T$_2$† for Glu and MI, similar caution should be employed when attempting to measure the concentration of these metabolites, giving consideration to the effects of J-coupling, MM contribution, and peak overlap, and how these all impact upon fit quality and reliability. As such in the present study, 40ms PRESS was chosen to be used for metabolite comparisons as previously has been shown to allow reliable detection of MI and Glu concentration with minimal overlap with surrounding peaks (Gasparovic et al., 2011; Mullins, Chen, Xu, Caprihan, & Gasparovic, 2008). Our results indicated no statistically significant metabolite concentration changes between younger and older participants, which is somewhat in contradiction to prior literature reports in regard to NAA, Cr, Cho, and MI. However, we did observe a trend towards a decrease in Glu within the older cohort compare to younger participants ($p < 0.037$, BF$_{+0}$ = 4.5). Age-related declines in Glu concentrations have been reported in the basal ganglia, parietal GM (Sailasuta, Ernst, & Chang, 2008), mesial motor cortex (Kaiser, Schuff, Cashdollar, & Weiner, 2005), left hippocampus, and cingulate cortex (Schubert, Gallinat, Seifert, & Rinneberg, 2004) at 3T and 4T. As Glu is proposed to be predominantly located in

neurons, a decrease in concentration levels may suggest neuronal alternations such as damage or loss (Kaiser et al., 2005), however one might also expect a decrease in NAA, another metabolite localised to the neurons. Of note is that our current measures for Glu are higher then normally reported but do fall between the levels reported for GM (22.06 m/mol) and WM (10.49 m/mol) in a previous paper (Gasparovic et al., 2011). The higher levels in our results may be a result of two factors – the first is the much shorter $T_2^\dagger$ we measured in our study than usually used, and the second may be a factor of the fitting software. While this makes some comparisons across studies difficult, for the purposes of a cross cohort within-study comparison these measures are still comparable. It should be mentioned that we have used a short TR in this experiment, which means that if T_1 had increased with age, it may mimic a decrease in Glu concentrations in much the same way that a decrease in $T_2^\dagger$ could have. However, any significant increase in T_1 would likely also be associated with an increase in $T_2^\dagger$, which we did not detect for Glu. Nevertheless, one should be aware of this possibility. Similarly one should be cautious in interpreting these results as we assume no change in water content within the tissues with age and this may not be correct. However, changes in water content would affect estimates of concentration for all metabolites, which we did not observe here, suggesting it has minimal impact on our results.

4.5.3. Medication effects

As life expectancy increases so does the use of medication treatment in the ageing population (Linjakumpu et al., 2002). The current study incorporated typically ageing older adults to be representative of the wider population in later life. Medication taken by older participants was to control for hypertension, thyroid dysfunction and cholesterol. Individuals with untreated chronic hypertensions have been linked to have similar increases of MI/Cr levels in the PCC as individuals with early AD compared to healthy age-matched controls (Catani et al., 2002). Furthermore, Haley et al. (2013) linked untreated hypertension, a mediator of obesity, to increased levels of MI/Cr in the occipitoparietal grey matter including the PCC. However, equally important to mention is research which proposed that medication treated hypertension may prevent dementia onset (Forette et al., 1998). Zhang et al. (2015) investigated untreated thyroid dysfunction in relation to metabolite changes in the PCC. The authors proposed significant increases of Glu concentration levels in hypothyroidism and decreases in hyperthyroidism compared to healthy controls. In the current study, the three older participants have taken medication to control their hypothyroidism. Interestingly, cholesterol is an essential component of brain function specifically in cellular membranes (Dietschy, 2009). Previous research has indicated that cholesterol in the body does not pass

the blood brain barrier (BBB), however cholesterol is converted to oxysterols, which in turn can pass the BBB (Gamba et al., 2015). Therefore, oxysterols are considered a potential link between high cholesterol, with involvement of additional factors such as oxidative stress, and the development of AD. Further, research has proposed that statins lowering cholesterol may lower the prospect of developing dementia (Panza et al., 2006). Due to the well-controlled hypertension, thyroid dysfunction and cholesterol along with the reviewed literature it is regarded that medication had minimal impact on metabolite concentration differences. However, the findings have to be carefully considered, even though the older adults have taken their medication regularly for over 4 weeks. Future research may consider exploring age-related medicated conditions in relation to neurometabolite differences across age and disease.

4.6. Conclusion

The findings highlight that metabolite quantification is a complex process where relaxation is an important factor to consider. This is especially important when examining metabolite concentration differences in ageing and disease. Alterations in metabolite concentrations may be misrepresented by changes in $T_2^{\dagger}$ relaxations and may lead to false evaluations of ^{1}H-MRS data.

4.7. Acknowledgements

Chapter 5

Glutamate and GABA Levels in Relation to Cognitive Performance Across Age

5.1. Abstract

Ageing is accompanied by cognitive changes as well as neurochemical alterations. The characterisation of neurochemistry and cognitive performance across age may further the understanding of changes across the lifespan. Here, we examined the concentrations for the major excitatory (glutamate) and inhibitory (GABA) neurotransmitters in relation to cognitive performance. Proton magnetic resonance spectroscopy was used to acquire glutamate and GABA concentrations from the posterior cingulate cortex (PCC) after the healthy younger (n = 19) and older (n = 20) adults completed cognitive assessments. The results showed older adults performing significantly worse on immediate and delayed visual reproduction as well as the Stroop interference task compared to the younger cohort. Age and reduced glutamate levels in the PCC were predictive of performance on immediate and delayed visual reproduction, while age was the only predictor of performance on Stroop interference task across both cohorts. We observed no GABA+ (plus macromolecules) correlation with cognitive performance across age. The findings indicate that age is certainly a substantial factor in cognitive performance; however, reduced Glu concentrations may play a subordinate role in cognitive performance.

Keywords: cognition, ageing, proton magnetic resonance spectroscopy, glutamate, GABA

5.2. Introduction

Age-associated decline in cognition has previously been shown in studies examining performance on cognitive tasks, predominantly on memory tests (Naveh-Benjamin & Kilb, 2014; Tulving, 1984). Older adults experience difficulties on verbal and non-verbal episodic memory tests (Tulving, 1984) as well as decreases in sensory acuity, processing speed, and spatial abilities (Naveh-Benjamin & Kilb, 2014; Pak, Czaja, Sharit, Rogers, & Fisk, 2008; Salthouse, 2000). However, there is limited knowledge on the neurophysiological underpinnings of cognitive change across the lifespan.

The in-vivo technique proton magnetic resonance spectroscopy (^{1}H-MRS) allows non-invasive measurement and monitoring of metabolites in the human brain (Ross & Bluml, 2001). Commonly, studied metabolites include *N*-Acetylaspartate (NAA), choline (Cho, which consists of choline, glycerophosphocholine, and phosphocholine), and creatine (Cr, which is a combination of creatine and phosphocreatine) along with the neurotransmitters glutamate (Glu) and γ-Aminobutyric acid (GABA) (Rae, 2014). ^{1}H-MRS studies have reported of mixed findings of metabolites changes across lifespan. A systematic review collating data from studies investigating age-related metabolite changes has observed no age-related alterations of NAA, Cho and Cr (Haga et al., 2009). However, a meta-analysis conducted on part of the studies (n = 4) suggested a trend for frontal NAA decrease as well as a significant increase in Cr and Cho concentration levels in the parietal region. In light of the authors' findings it appears that concentrations are in part dependent on brain location and tissue content as well as the ratio that metabolites are referenced to. More recent research suggests an increase in Cr concentrations in the posterior cingulate cortex (PCC), while Cho/Cr ratio is observed to decrease with age (Reyngoudt, et al., 2012). In comparison, a study investigating the PCC as well as the anterior cingulate cortex (ACC) found increased concentration levels of Cho and Cr with age (Chiu et al., 2014). There is limited research investigating Glu and GABA, the primary excitatory and inhibitory neurotransmitters in the central nervous system. Age-related Glu decrease, however, has been reported in the hippocampus, ACC, grey matter motor cortex region and striatum (Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013; Kaiser, Schuff, Cashdollar, & Weiner, 2005; Zahr, Mayer, Pfefferbaum, & Sullivan, 2008). Research investigating age-related GABA+ (plus macromolecules) changes has reported of a decline in frontal and parietal brain regions (Gao et al., 2013) while a trend for an increase in GABA+ has been observed in the ACC, (however the authors caution that the increase may be driven by macromolecules) (Aufhaus et al., 2013).

Only a few studies have attempted to link cognitive process with brain metabolites (Ferguson et al., 2002; Zahr et al., 2013). Higher parietal cortex NAA/Cr and Cho/Cr ratios have been shown to correlate positively with a better performance on memory test such as Logical Memory, delayed 24h Logical Memory and Verbal Memory Factor, in healthy elderly men (Ferguson et al., 2002). Additionally, Cho/Cr has been positively linked with the memory tests: Visual Reproduction; Visual Retention Test; and Auditory-Verbal Memory Test. Conversely, age-related reduction in striatal Glu levels has been reported to correlate positively with poor performance on fluency and working memory, while a negative correlation was observed between lower striatal Glu levels and set shifting (Zahr et al., 2008). In a further study, the authors reported of a positive correlation between decreased striatal Glu levels and declined performance on Grooved Pegboard (Zahr et al., 2013).

The current study examines the PCC, which has a central role in the default mode network (DMN) with high activity at rest (Raichle et al., 2001). This region of interest (ROI) is well connected with other brain areas such as medial temporal lobes and retrosplenial cortex, which overlap with memory systems (Greicius, Supekar, Menon, & Dougherty, 2009; Squire, Stark, & Clark, 2004). Interestingly, research has implicated the PCC with episodic memory processing and autobiographical memory retrieval (Dunn et al., 2014; Maddock, Garrett, & Buonocore, 2001). In the present study we tested the following hypothesis: higher Glu and GABA concentration levels will be predictive of cognitive performance (higher scores) on neuropsychological assessment across age in the PCC. Supplementary analyses have been taken of age and gender effects across age.

5.3. Method

5.3.1. Participants

Participants were healthy 19 younger (9 females, aged 20 - 27, M = 22.32 ± 2.3 years) and 20 older (10 females, aged 56 - 84, M = 67.70 ± 7.97 years) adults from the student population and local community. The random sample was recruited through flyers, the Bangor School of Psychology research participant panel, community facilities (such as churches and supermarkets), and word of mouth. Inclusion criteria were: normal to corrected-to-normal vision and hearing; fluency in English due to the nature of neuropsychological assessment; age cut-offs for younger and older adults 18 to 30 and 55 to 90 years, respectively; and MMSE total score cut-off of 24 to 30. For all participants, the exclusion criteria included self-reported medication usage (except contraception for females)

along with history of neurological and psychiatric disorders in conjunction with MRI safety screening form criteria. All experimental protocols were approved by Bangor University School of Psychology Ethics and Research Committee (2013-11044-A12103). Participants received a study information sheet and had the opportunity to ask questions before providing written informed consent to take part in the study. Monetary compensation was provided to participants for their time along with debriefing and the opportunity to ask any additional questions at the end of the session.

5.3.2. Neuropsychological assessment

All participants underwent a one-hour battery of neuropsychological assessments, with a break half way through to avoid fatigue, before a one-hour scan. Participants were screened for cognitive deficits as well as mood with the Mini Mental State Examination (MMSE, Folstein, Folstein & McHugh, 1975) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983), respectively. To assess immediate and delayed auditory and visual memory the following standardized tests were implemented: the Wechsler Memory Scale – Third Edition (WMS-III, Wechsler, 1997) subtests: Logical Memory and Visual Representation. The Stroop Neuropsychological Screening Test (SNS, Trenerry, Crosson, DeBoe, & Leber, 1989) was administered to assess executive functions, specifically, inhibition.

All participants scored (younger: $M \pm SD = 28.2 \pm 1.7$; older: $M \pm SD = 28.7 \pm 1.5$) within the recommended normal range for cognition on the MMSE. Education did not differ significantly between the two cohorts. Two younger participants were excluded as a result of their scores falling into the moderate and severe range on the anxiety subscale. Table 1 displays demographics and assessment mean scores for all study participants. Individuals within the mild range (8-10) for anxiety ($n = 5$) were not excluded, as their scores were considered marginal (8) and possibly influenced by assessment and scanning.

	Younger (n=19) (Mean $\pm$ SD)	Older (n=20) (Mean $\pm$ SD)	*P* values
Age	22.32 ± 2.31	67.70 ± 7.97	< .001
Gender	M 10 / F 9	M 10 / F 10	.874
Education	16.47 ± 1.22	15.65 ± 3.41	.326
HADS – Anxiety	4.79 ± 1.90	4.60 ± 2.70	.803
HADS – Depression	2.16 ± 1.86	1.85 ± 2.11	.633
MMSE	28.16 ± 1.70	28.65 ± 1.46	.339
Stroop task	109 ± 8.15	97.50 ± 14.40	.004
Immediate memory	44.84 ± 9.65	40.10 ± 13.35	.210
Delayed memory	29.68 ± 6.54	25.80 ± 10.27	.166
Immediate visual reproduction	93.26 ± 10.03	79.65 ± 14.93	.002
Delayed visual reproduction	88.95 ± 17.54	59.50 ± 18.97	< .001

Table 1. The basic demographics and cognitive performance mean scores of younger and older participants.

5.3.3. ^{1}H-MRS acquisition and processing

All scans were performed on a 3T Philips Achieva MR scanner (Philips Health Care, Eindhoven, Netherlands) with a SENSitivity-Encoded (SENSE) 32-channel head coli. Structural images (T$_1$-weighted; MP-RAGE; FOV = 220 x 220 x 180mm^3; TE = 5ms; TR = 21ms; slice thickness = 1mm; flip angle = 8°) were obtained for quantification purposes of CSF and tissue volumes as well as placement of the ^{1}H-MRS voxel in the PCC.

The metabolite GABA requires a special acquisition technique, as it has three coupled resonances at 1.9ppm, 2.28ppm, and 3.01ppm, which overlap with NAA/ NAAG, Glu/ Gln, and Cr, respectively. The MEGA-PRESS (MEscher-Garwood Point RESolved Spectroscopy; Mescher et al., 1996, 1998) editing technique is the most commonly used method for detection of GABA (Mullins 2014) and consists of two acquisitions, one 'ON' and one 'OFF'. During the 'ON' acquisition an additional editing-pulse is applied to the resonance peak at 1.9ppm (parts per million) in a modified PRESS sequence. This makes use of the J-coupling of the peak at 1.9 ppm with the peak at 3.01ppm (Mescher et al., 1998; Mullins et al., 2014),

leading to a refocus of the evolution of the J-coupling to the 3.01 peak. The OFF spectrum is collected with a similar additional rf pulse, in a symmetrical position around the water peak (usually at 7.5 ppm). By subtracting the 'ON' from the 'OFF' acquisition spectra most metabolite peaks cancel out, leaving an edited GABA peak, as well as a downward phased NAA peak, and combined peaks for glutamate and glutamine, denoted as Glx. Due to a potential contribution from co-edited macromolecules (MM) at 3.01ppm, GABA will be referred to as GABA+ in the rest of the manuscript.

Single voxel ^{1}H-MRS was performed using MEGA-PRESS in the PCC with a voxel size of 25 x 25 x 25mm (Fig. 1). The following parameters were used for spectral acquisition: TR/TE = 2000/ 80ms; averages = 320; phase cycles = 16; spectral width = 2000 Hz; shim voxel size = 35 x 35 x 35mm). To suppress the water signal CHEmical Shift Selective (CHESS) pulses were applied. Additionally, an unsuppressed water scan was acquired of the same voxel, in order to use brain water concentration as normalization for metabolite concentrations.

All spectral analsyis was performed using TARQUIN 4.3.8 (Wilson, Reynolds, Kauppinen, Arvanitis, & Peet, 2011). GABA measure were obtained from the edited spectrum, which was fit using the MEGA-PRESS GABA basis set and options in Tarquin. Glutamate measures however were fit from the OFF edited spectrum as a standard PRESS acquisition. This PRESS spectrum was fit to a simulated basis in which the following metabolites were included in the model: alanine (Ala), aspartate (Asp), creatine (Cr), γ-aminobutyric acid (GABA), glucose (Glc), glutamine (Gln), glutamate (Glu), glycerophosphorylcholine (GPC), glycine (Gly), guanidinoacetate (Gua), myo-inositol (MI), lactate (Lac), N-acetyl-aspartate (NAA), N-acetyl-aspartylglutamate (NAAG), phosphorylcholine (PCh), phosphocreatine (PCr), scyllo-inositol (s-Ins), taurine (Tau), as well as models for the macromolecular and lipid components. The data were fit with the following processing parameters: dynamic averaging (WS) = average all scans; dynamic averaging (W) = average all scans; dynamic correction reference signal (WS) = 1H Cr Cho; residual water removal with cut of at 45 Hz; automatic phasing and referencing; water concentration of 55.5 mol/l and line-broadening of 4 Hz for visualization purposes.

CSF and tissue volume segmentation were determined using an in-house partial volume correction code to correct for partial volume effects. Transverse (T_2) relaxation values for NAA, Cr, Cho, Glu and MI were derived from Chapter's 3 and 4 findings. Literature longitudinal (T_1) relaxation values for metabolites and water were incorporated to correct for relaxation effects (Table 2).

	Glu	GABA	NNA	Cho	Cr	MI	Water (GM)	Water (WM)	CSF
T_1	1220	131	1403	1182	1320	1102	1488	781	4000
T_2	169	88	247	254	160	200	71	58	200

Table 2. Displayed are mean literature T_1 values for metabolites and water as well as water T_2 values in ms. Metabolite T_2 values (except of GABA, which are literature based) are a combination of literature (Chapter 3) and Chapter 4 mean T_2 values.

5.3.4. Statistical analysis

IBM SPSS Statistics version 22 (IBM Corp. in Armonk, NY) was used for all statistical analyses and is reported here as two-tailed. Age-related Glu and GABA+ differences were assessed with independent samples t-tests. Bonferroni correction was applied to correct for multiple comparison (2 metabolites) while the level of significance was set to $p < .025$. Age-related cognitive performance differences were determined with independent samples t-tests, whereas the level of significance was set to $p < .008$ using Bonferroni correction to correct for multiple comparisons (6 tests). Multiple linear regression analyses were performed to assess the relationship between age and PCC metabolite concentration levels (Glu and GABA+) with cognitive performance (raw scores of STROOP test, WMS-III Logical memory and visual representation - immediate and delayed) across age with a p-value set at $p < .01$ (5 tests). A secondary analysis was conducted on NAA, Cr, Cho, and MI across age, which enclosed independent samples t-tests with a corrected p-value set a $p < .0125$ (4 tests). Subsequently, a partial correlation was applied to investigate the relationship between Glu and GABA with cognitive performance, while controlling for age.
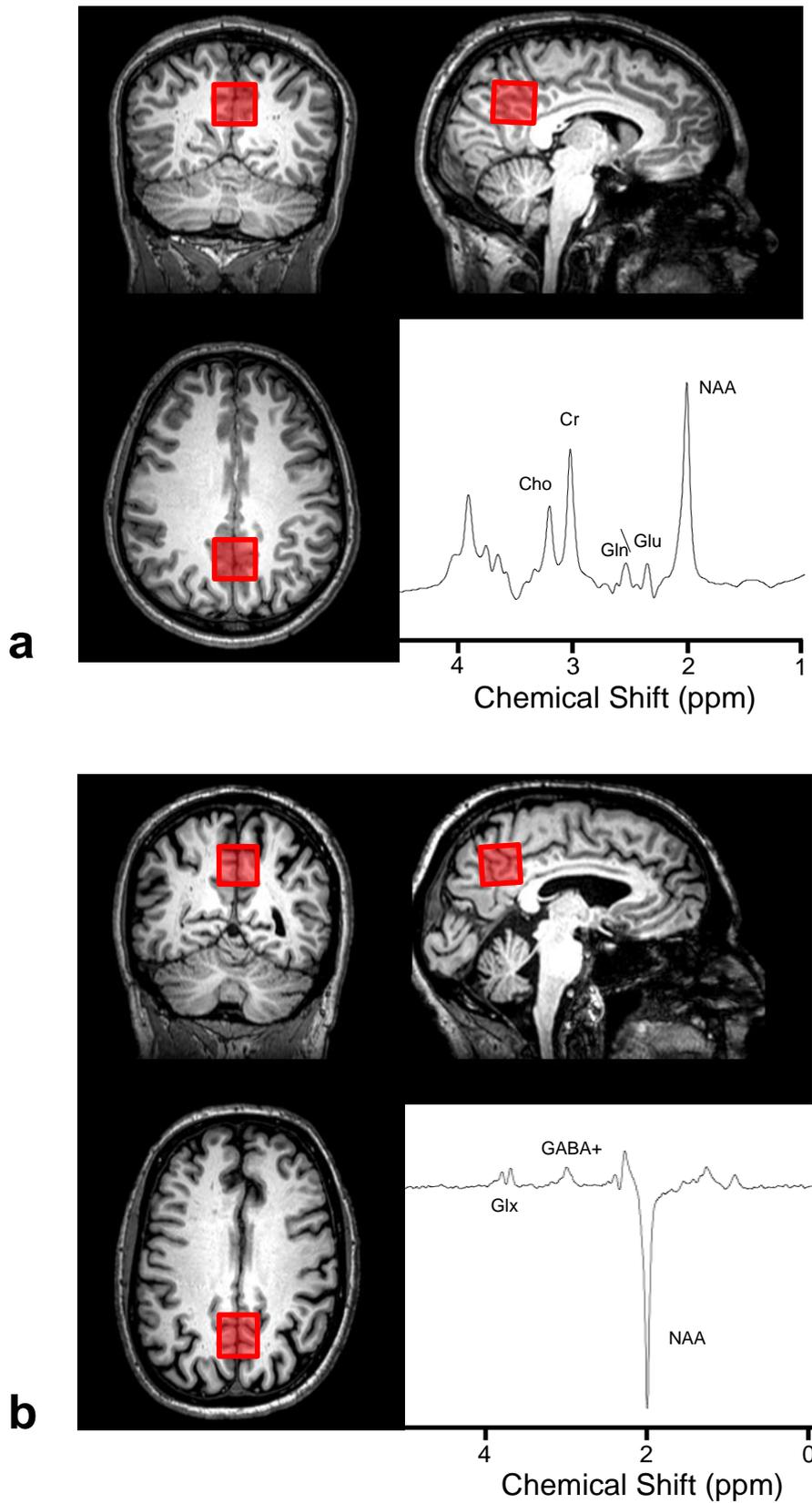
Figure 1. ¹H-MRS voxel was placed in the PCC represented by a sample from a younger (a) and older (b) adult. Sample spectrum (a) represents PRESS sequence used for Glu analysis, while spectrum (b) represents MEGA-PRESS sequence for GABA analysis.

5.4. Results

5.4.1. Age effects on metabolites

We observed a significant decrease of Glu concentration levels ($t(37) = 3.67$, $p < .001$) in older ($M \pm SD = 10.59 \pm 2.10$) compared to younger ($M \pm SD = 12.79 \pm 1.60$) participants in the PCC (Fig. 2). No significant age-related differences were observed of GABA+ ($p = .22$) concentration levels (Younger: $M \pm SD = 1.11 \pm .15$; Older: $M \pm SD = 1.16 \pm 0.14$).

For the secondary analysis only a trend was observed for NAA ($p = .03$) concentration levels across age, while Cr, Cho, and MI were not significantly different with age.
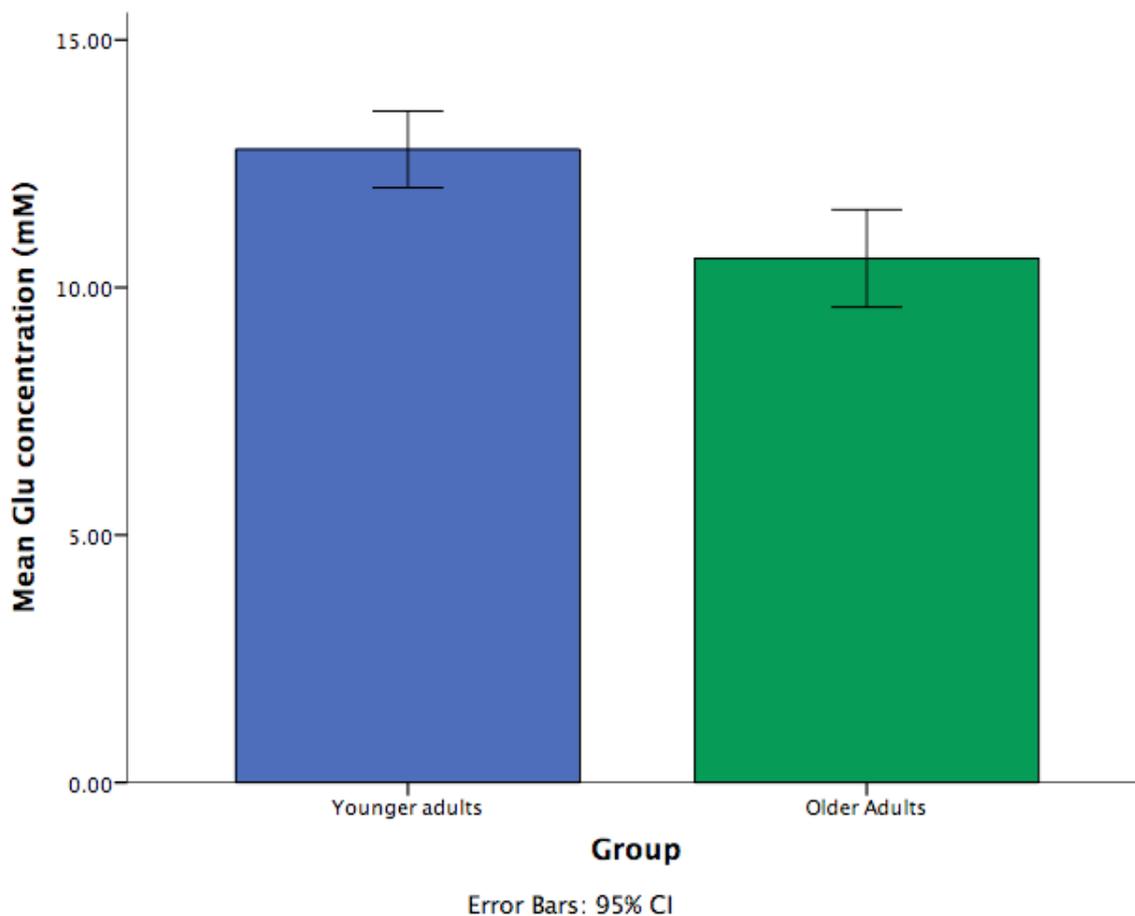
Figure 2. The mean PCC Glu concentration levels are displayed for younger ($M \pm SD = 12.79 \pm 1.60$) and older ($M \pm SD = 10.59 \pm 2.10$) participants.

5.4.2. Age effects on cognitive performance

Older participants ($M \pm SD$ = 97.50 $\pm$ 14.41) performed significantly poorer on the Stroop task (t(37) = 3.09, p = .004) compared to younger participants ($M \pm SD$ = 109 $\pm$ 8.15) (Fig. 3). Performance on immediate (p = .21) and delayed (p =.17) logical memory recall showed no significant age-related differences between both cohorts. However, the learning slope on immediate auditory memory recall was significantly higher (t(37) = 2.91, p = .006) for younger ($M \pm SD$ = 6.58 $\pm$ 2.34) compared to older ($M \pm SD$ = 4.35 $\pm$ 2.43) cohort (Fig. 4). Older participants performed significantly worse on immediate (t(37) = 3.32, p = .002) (Fig. 5) and delayed (t(37) = 5.03, p < .001) visual reproduction compared to younger participants (Fig. 6).

Error Bars: 95% CI
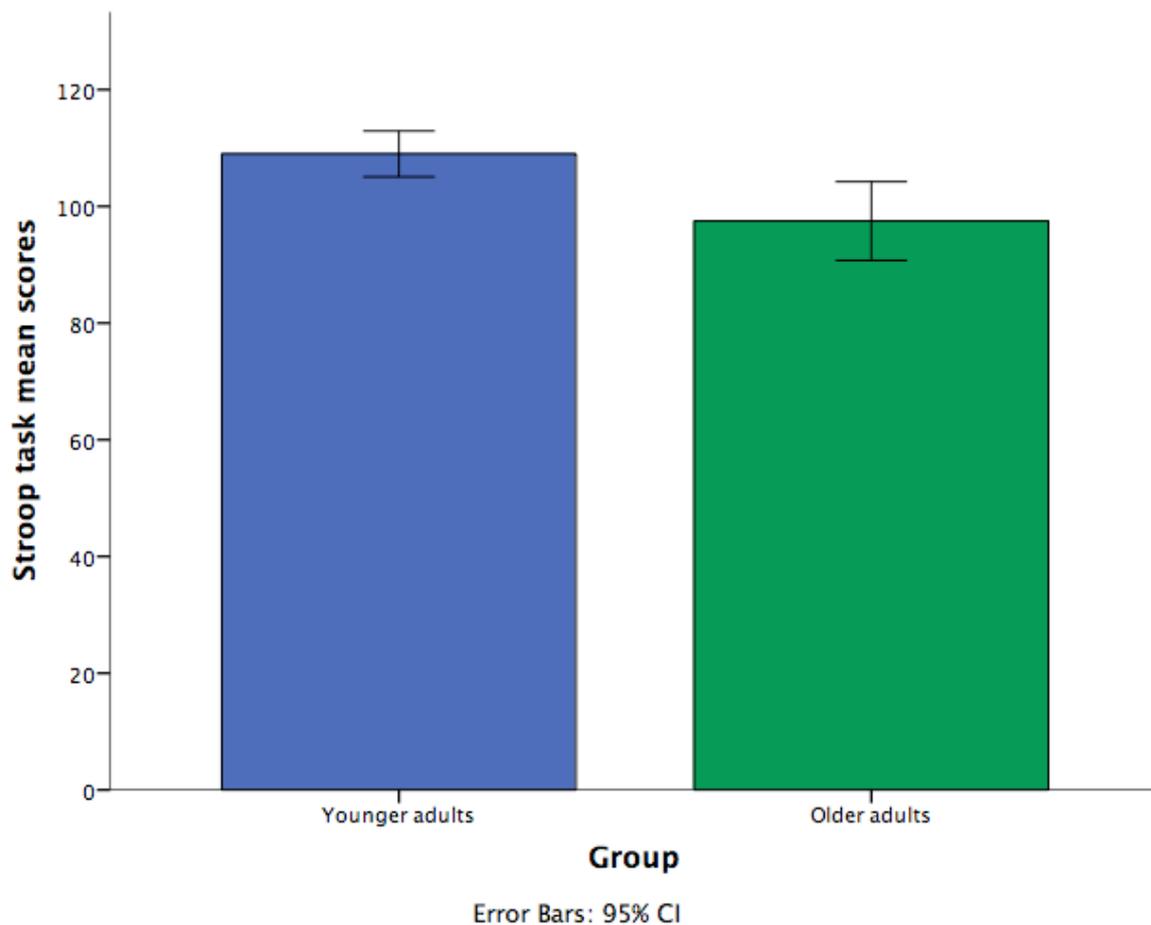
Figure 3. Stroop task mean scores for younger ($M \pm SD$ = 109 $\pm$ 8.15) and older participant ($M \pm SD$ = 97.50 $\pm$ 14.41) displaying poorer performance in older cohort.
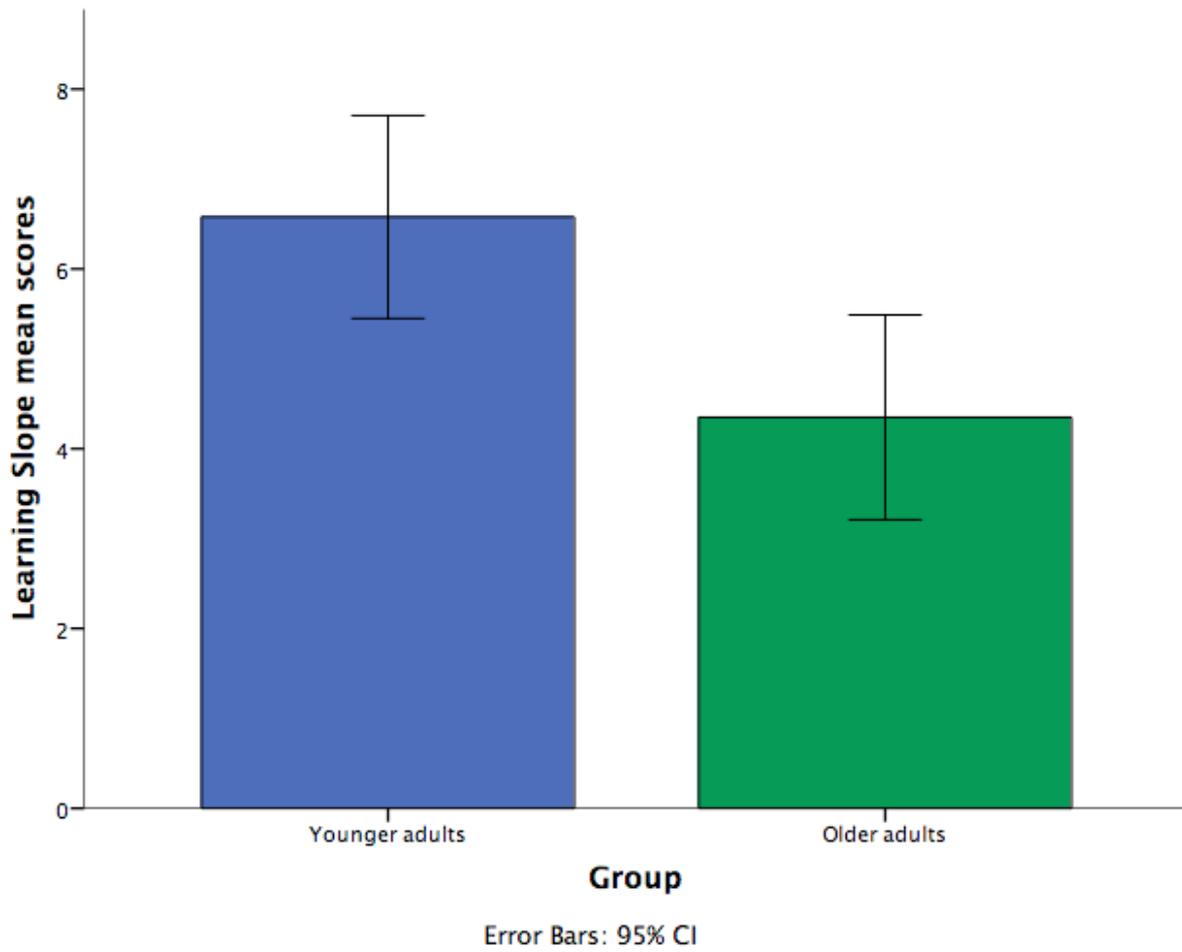
Figure 4. Displayed are mean learning slope scores on immediate auditory memory recall for younger ($M \pm SD = 6.58 \pm 2.34$) and older ($M \pm SD = 4.35 \pm 2.43$) adults.
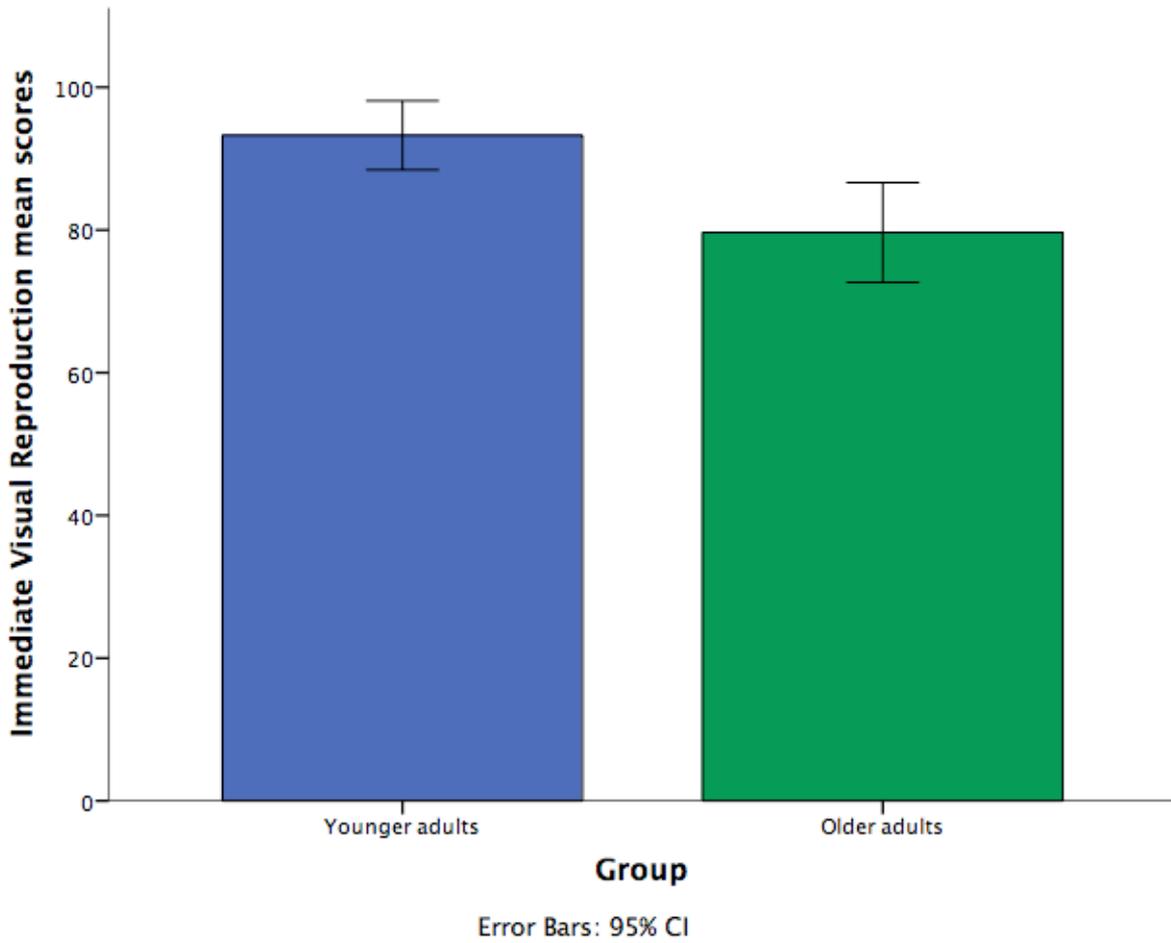
Figure 5. Presented are immediate visual reproduction mean scores for younger (*M* ± *SD* = 93.26 ± 10.03) and older (*M* ± *SD* = 79.65 ± 14.94) participants.
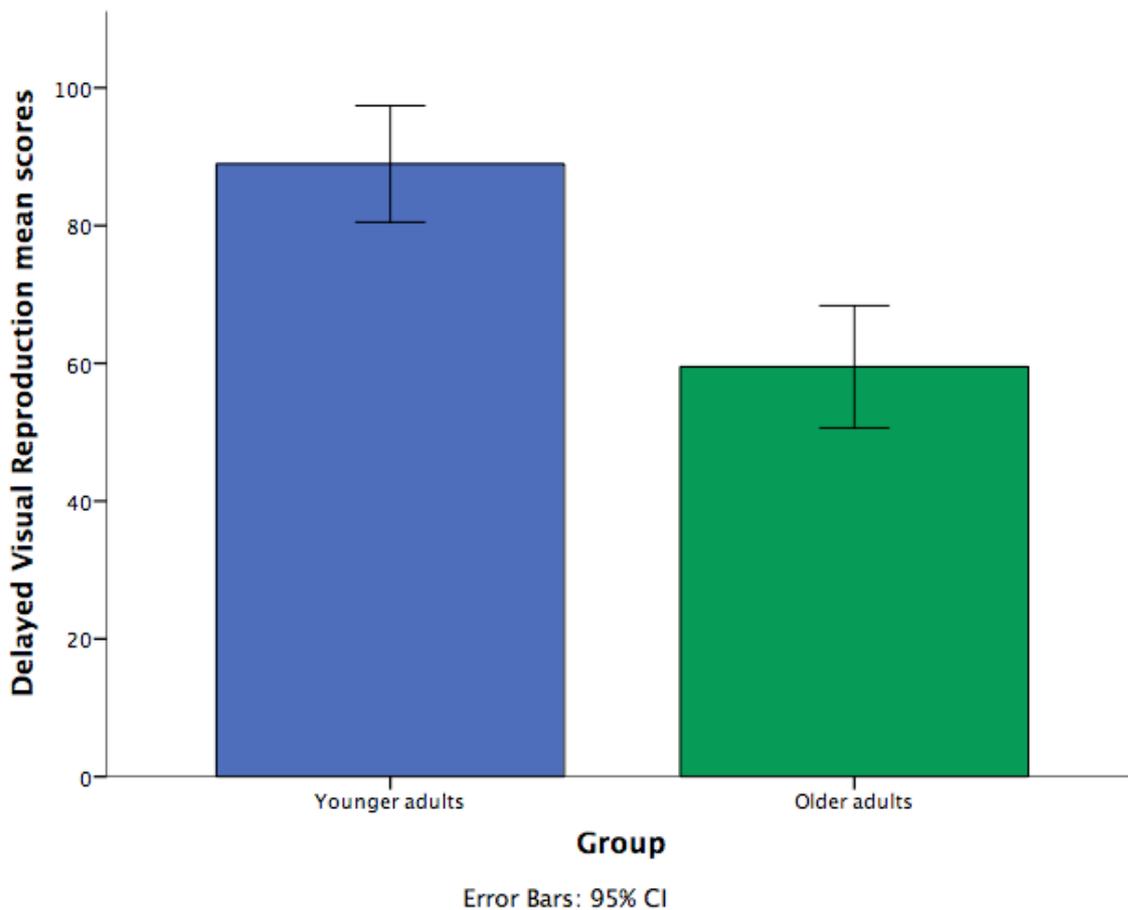
Figure 6. Displayed are delayed visual reproduction mean scores for younger (*M ± SD =* 88.95 ± 17.54) and older (*M ± SD* = 59.50 ± 18.96) cohort.

5.4.3. Relationship between metabolites and cognitive performance

For the Stroop task, a multiple-regression model including age, Glu, and GABA together accounted for 29% of the variance in performance ($F(3,35) = 4.73$, $p = .007$) in both cohorts. Of the three variables, age was the largest unique contributor (beta =-.66, $p = .002$).

A multiple-regression model of immediate visual reproduction revealed that age, Glu, and GABA+ contributed 36% of the variance in performance ($F(2,35) = 6.48$, $p = .001$) across both cohorts. Of the three variables, age was the largest unique contributor (beta =-.46, $p = .013$). When age was removed from the model, Glu and GABA contributed 23% of the variance in performance ($F(2,36) = 5.39$, $p = .009$). Of the two variables, Glu made a significant unique contribution with a higher beta value (beta =.50, $p = .002$, Fig. 7) than

GABA+ (beta =-.11, $p = .49$). Age, Glu, and GABA+ contributed 52.8% of the total variance of performance on delayed visual reproduction ($F(2,35) = 13.06$, $p < .001$), while age was the largest unique contributor (beta =-.72, $p < .001$). However, when age was removed from the model, Glu and GABA contributed 21.8% of the total variance of performance on delayed visual reproduction ($F(2,36) = 5.03$, $p = .012$). In this model, Glu made a significant unique contribution with a higher beta value (beta = .47, $p = .004$, Fig. 8) than GABA+ (beta =-.03, p =. 87). Examination of the models has revealed no violation of the assumptions of multicollinearity, normality, linearity, and homoscedasticity. Further multiple-regression models did not withstand correction for multiple comparisons.

Additionally, partial correlation was used to assess Glu and GABA concentrations in relation to cognitive performance, while controlling for age. The outcome showed no significant correlations between Glu and GABA with cognitive performance.
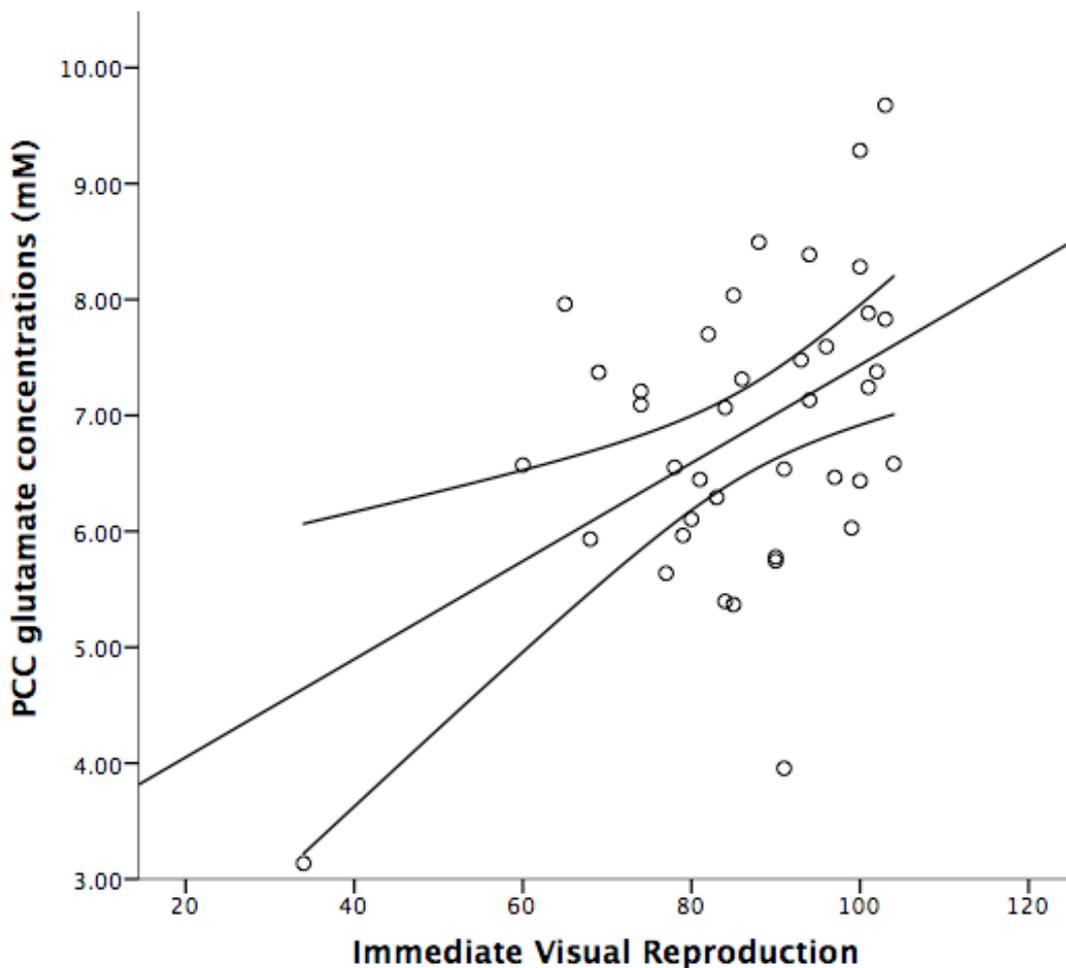
Figure 7. Scatterplot of multiple regression between PCC Glu and immediate visual reproduction across both cohorts.
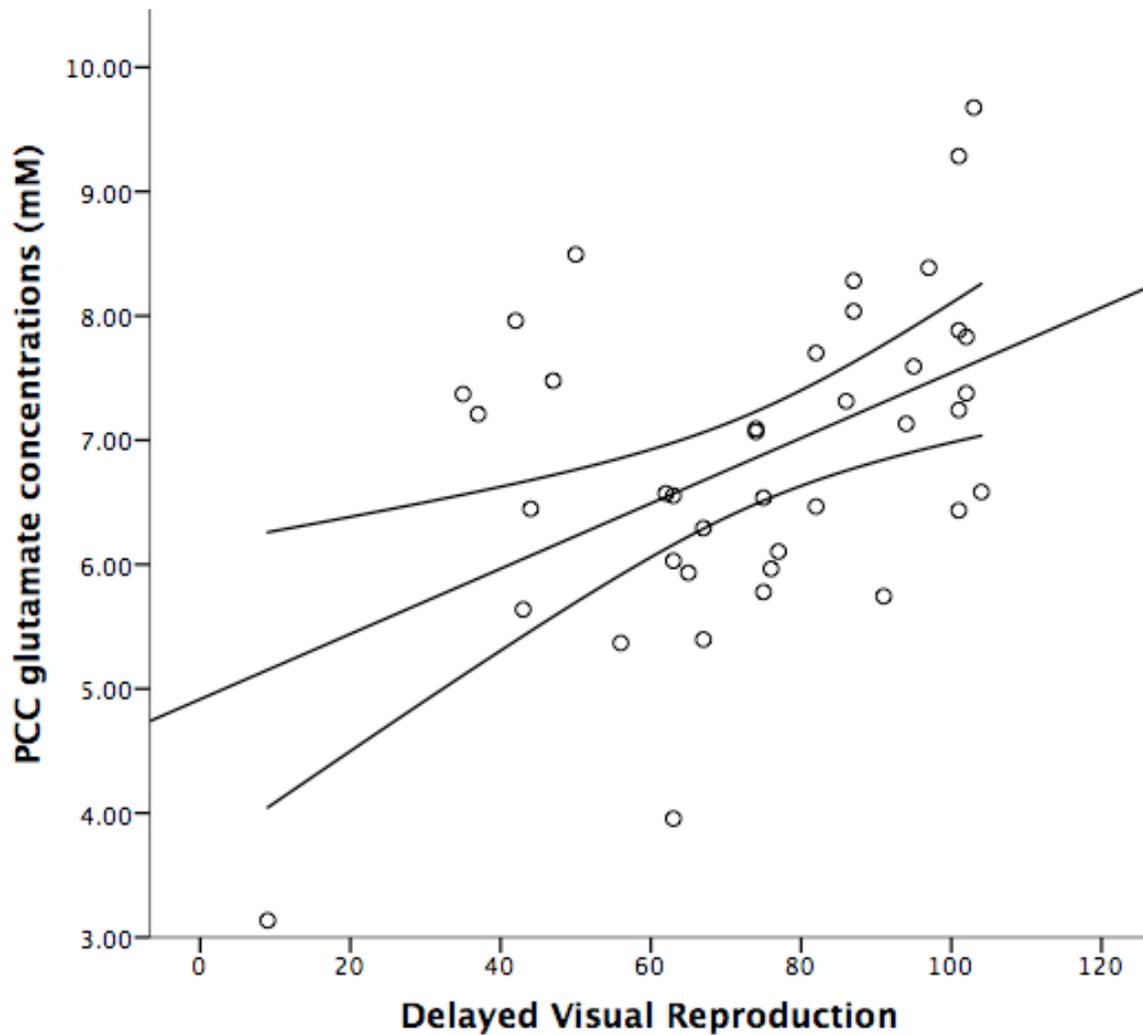
Figure 8. Scatterplot of multiple regression between PCC Glu and delayed visual reproduction across both cohorts.

5.4.4. Gender effects

Independent samples t-tests were used to review if any gender differences exist in Glu and GABA+ concentration levels (significance level set to $p < .0125$). The results showed a significant increase ($t(17)= 3.17$, $p = .006$) in GABA+ concentration levels in younger females ($M \pm SD = 1.13 \pm 0.12$) compared to younger males ($M \pm SD = .97 \pm .11$). This has not been observed in the older cohort between genders for either Glu or GABA+.

5.5. Discussion

The ageing process is accompanied by a diversity of mechanisms such as cognitive and neurochemical changes. As a result, ageing is commonly considered a primary risk factor for various cardiovascular and neurodegenerative diseases such as dementia (Lockhart & DeCarli, 2014; Tomasi & Volkow, 2012). The characterisation of neurochemistry and cognitive performance across age may further the understanding of changes across the lifespan. The current study concentrated on the neurotransmitters Glu and GABA+ in conjunction with cognitive performance across age. The main findings suggest age and reduced Glu levels in the PCC are predictive of performance on the immediate and delayed visual reproduction recall, while age is only predictive of performance on the Stroop interference task across both cohorts. GABA+ showed no relation to cognitive performance.

5.5.1. Age effects on metabolites

Our finding of age-related decline in Glu concentration changes in the PCC corresponds with previous literature. Earlier studies have reported of Glu decreases in the ACC, hippocampus, striatum and grey matter cortex (Hädel, Wirth, Rapp, Gallinat, & Schubert, 2013; Kaiser, Schuff, Cashdollar, & Weiner, 2005; Zahr, Mayer, Pfefferbaum, & Sullivan, 2008). Glu is predominately found in neurons compared to glia cells with higher concentration in the intracellular as to extracellular space (Mark et al., 2001). It was proposed that a potential decrease in Glu might be indicative of damage or shrinkage in neuronal integrity (Kaiser et al., 2005), however a secondary analysis on the remaining metabolites only showed a trend in NAA decrease with age. There were no age-related changes in GABA+ concentration levels in the PCC. A previous study has reported of a decline in GABA+ concentrations levels in frontal and parietal brain regions (Gao et al., 2013). While another study observed a trend in increase of GABA+ but no change in GABA across age in the ACC (Aufhaus et al., 2013). It was suggested that macromolecules rather than GABA itself might drive the increase. As a result, there are only a few studies in this research area and further research is required to explore potential differences in GABA+ and GABA across the brain and with age.

5.5.2. Age effects on cognitive performance

Our findings of older adults performing worse on the Stroop task compared to younger adults, is in agreement with previous published literature (Houx, Jolles, & Vreeling, 1993;

Spieler, Balota, & Faust, 1996). Older individuals experience the Stroop interference effect with an increased reaction time and error rate on naming the incongruent colour words compared to younger individuals (Davidson, Zacks, & Williams, 2003). Interestingly, we have not observed any differences in performance on immediate and delayed logical memory recall but only on the immediate and delayed visual reproduction between cohorts. Research in this field has shown that performance on immediate and delayed memory tasks declines with age (Haaland, Price, & Larue, 2003). Haaland et al. (2003) suggested that age-related changes on immediate and delayed recall might in fact reflect age-related issues with encoding and retrieval rather than storage. It is possible that our older cohort's age was too broad. Previous research has suggested that increased reduction in performance was shown on test such as visual memory or facial recognition in older adults. While a decline in performance on tests measuring memory or visuoperception sets on around 80 years of age and onwards (Benton, Eslinger, & Damasio, 1981).

5.5.3. Relationship between metabolites and cognitive performance

The results have shown age as a significant predictor of performance on the Stroop interference task across both cohorts. This outcome is in line with several other studies reporting that cognitive performance is largely affected by age (Cohn, Dustman, & Bradford, 1984; Verhaeghen & De Meersman, 1998). As such, age has also a negative impact on sensory acuity, processing speed, and spatial abilities (Naveh-Benjamin & Kilb, 2014; Pak, Czaja, Sharit, Rogers, & Fisk, 2008; Salthouse, 2000).

Further, the findings revealed that age, as the major contributor, along with decreased PCC's Glu levels were significant predictors of performance on immediate and delayed visual reproduction across both cohorts. Previous research has reported comparable findings of reduced Glu levels predicting poorer performance on cognitive tasks (Zahr et al., 2008; Zahr et al., 2013). As such, Glu has also been linked to memory and learning through research into Glu receptors mediating signal pathways (Peng, Zhang, Zhang, Wang, & Ren, 2011). However, given that the majority of Glu detected in ^{1}H-MRS is intra-cellular and not directly involved in neurotransmission, it is difficult to make a direct link between Glu levels and cognitive performance. Notably, it has been reported that the metabolic pool contains 70-80% of tissue Glu, while glutamatergic nerve terminals only hold 20-30% (Fonnum, 1993). While some researchers have used Glu levels as proxies for excitatory potential (Stagg et al., 2009), others suggest it is more likely a measure of global metabolism in the region (Bednařík et al., 2015; Mangia et al., 2009). Considering the later, the reduction in Glu in our

older cohort within the PCC may reflect reduced metabolism, or neural activity in this region, which may also explain the correlation to performance on our cognitive tests. Research has suggested that changes in metabolic activity may be linked to decreases in Glu concentrations (Fonnum, 1993). It would be interesting to investigate basal blood flow (as another proxy for activity levels) in these regions as well. That aside, when controlled for age, Glu concentrations and cognitive performance on immediate and delayed visual reproduction result in a non-significant correlation. Separately however, Glu concentrations have been found to decline with age, as does cognitive performance decrease with age. It is unclear if cognitive performance is governed by a general age effect or by Glu concentration alterations. It is possible that a decline in cognitive performance occurs prior to a decrease in Glu concentration levels or vice versa. There was no relation observed between GABA+ and cognitive performance, although research investigating GABA(A) receptor, utilizing gene disruption, has previously suggested links with learning and memory in animal studies (Collinson et al., 2002).

5.5.4. Gender effects

Previous MRS research has suggested gender differences in Glu and Gln, and GABA+ concentrations (De Bondt, De Belder, Vanhevel, Jacquemyn, & Parizel, 2015; Epperson et al., 2002). Therefore, we found it essential to examine any gender differences in our data. The present findings only suggest gender differences in GABA+ concentration levels in younger but not older cohort. Younger females had significantly less GABA+ in the PCC compared to younger males. There were no Glu gender specific differences across age. It has been reported that GABA+/Cr concentrations are higher in females during ovulation phase compared to the other menstrual cycle or contraception phases (De Bondt et al., 2015). This may indicate why there were no gender differences in the older cohort, especially, if older females have passed their menopause. However, further research is required to expand on this topic. As female participants were not required to provide their menstrual or contraceptive status, this should be considered as a limitation of the present study.

5.5.5. Limitations

There are several limitations of the present study, which should be considered. First, our older cohort had a broader age range compared to the younger cohort. Previous studies have also used a broader age range, however it would be sensible to investigate

neurochemical changes in relation to cognition according to age by decade, as chronological age may be a poor indicator of the onset of ageing process (Segovia et al., 2001). This may provide an improved representation of age-related changes in regard to neurochemical and cognitive processes.

Consideration has to be taken when interpreting the results due to the compartmentation of Glu in the brain (Fonnum, 1993). As previously mentioned the detected Glu concentration is mostly intra-cellular and not directly involved in neurotransmission. Further, ^{1}H-MRS detection of Glu cannot directly differentiate between the intra- and extra-cellular changes; therefore, care has to be taken when defining relations between Glu concentrations and cognitive performance.

As previously mentioned females were not required to provide their menstrual status, therefore, our data should be considered with caution as GABA+ concentration differences were observed between females and males in the younger cohort.

5.6. Conclusion

In brief, the data identified that Glu decreases with age, and that age and decreased Glu levels in the PCC are significant predictors of cognitive performance. Future studies into ageing's effects on cognition and metabolism would benefit from the use of a multimodal approach to investigate the links between neurochemical and cognitive changes across the lifespan.

5.7. Acknowledgments

Chapter 6

Resting-state glutamate and GABA in relation to functional connectivity interrelations across age

6.1. Abstract

Research has suggested that glutamate (Glu) and γ-Aminobutyric acid (GABA) concentrations modulate blood-oxygenation-level dependent (BOLD) activity in the human brain. As such, we investigated the relation of Glu and GABA+ (plus macromolecules) with the functional connectivity between posterior cingulate cortex (PCC) and hippocampus across age. Previously, it was reported that individuals with mild cognitive impairment, a pre-stage to Alzheimer's disease, experience abnormal connectivity between the PCC and hippocampus. We measured Glu and GABA+ concentrations in the PCC utilizing proton magnetic resonance spectroscopy (¹H-MRS) and functional connectivity between PCC and hippocampus using functional magnetic resonance imaging (fMRI) in 39 healthy younger and older adults. The result suggests a positive correlation between resting-state Glu concentrations and functional connectivity between PCC and hippocampus across age, while there was no significant correlation with GABA+ concentrations. These findings are supportive of the concept of glutamatergic involvement in functional connectivity in the human brain.

6.2. Introduction

Given the increasing knowledge on cognitive processes across lifespan, there is still limited research on physiological changes. Proton magnetic resonance spectroscopy (¹H-MRS) and functional magnetic resonance imaging (fMRI) are techniques, which can further knowledge of cognitive processes by obtaining measure of different physiological information regarding neuronal activity.¹H-MRS takes advantage of the differing magnetic environments for atomic nuclei on different molecules to obtain metabolite concentrations, while the commonly used blood-oxygenation-level dependent (BOLD) fMRI contrast measures blood susceptibility changes as a result of resting-state or task-based activity (Bandettini, 2012; De La Iglesia-Vaya, Kanaan, Molina-Mateo, Martí-Bonmati, & Escarti-Fabra, 2013; Hore, 2015).

The major excitatory and inhibitory neurotransmitters, glutamate (Glu) and γ-Aminobutyric acid (GABA), respectively, have been indirectly linked to BOLD signal activity (Buzsáki, Kaila, & Raichle, 2007). This relation has been examined in relation to BOLD signal activity in both resting-state and task-based fMRI studies. Previous research reports that measures of resting state GABA concentrations have an inverse correlation to task-based BOLD signal in the visual cortex (Stagg, Bachtiar, & Johansen-Berg, 2011), medial occipital cortex (Muthukumaraswamy, Edden, Jones, Swettenham, & Singh, 2009), and primary motor cortex (Donahue, Near, Blicher, & Jezzard, 2010). Another negative correlation has been observed between resting GABA/ Cr+PCr ratio in the PCC and the connectivity strength of putamen to the default mode network (DMN) (Arrubla et al., 2014). Similarly, it has been reported that DMN intrinsic functional connectivity is positively correlated with Glu/Cr ratio, while the network experiences a negative correlation with GABA/Cr ratio (Kapogiannis, Reiter, Willette, & Mattson, 2013). Moreover, the relation between Glu concentrations and task-based BOLD signal has been reported to be dependent on task demands (Falkenberg, Westerhausen, Specht, & Hugdahl, 2012). The observations in these studies have mainly focused on single groups and task-based BOLD activity, as such it is unclear if there are alterations across age.

The target regions, posterior cingulate cortex (PCC) and hippocampus have been identified as key regions in the DMN (Buckner, Andrews- Hanna, & Schacter, 2008). Regions that have also been identified to be part of the DMN include: ventral medial prefrontal cortex, retrosplenial cortex, inferior parietal lobule, lateral temporal cortex, and dorsal medial prefrontal cortex. The PCC is considered the hub of the DMN and experiences high activity during rest (Raichle et al., 2001), while the hippocampus is involved in memory processes

(Eichenbaum, 2001). Reduced DMN activity has been observed in older compared to younger individuals as well as reductions in memory functions (Cabeza, et al., 2004; Damoiseaux et al., 2008). Based on previous findings the DMN regions overlap with the activity of medial temporal lobe during memory functions (Squire, Stark, & Clark, 2004). Furthermore, the PCC has been implicated with autobiographical memory retrieval and episodic memory processing (Dunn et al., 2014; Maddock, Garrett, & Buonocore, 2001). In this context, the authors (Dunn et al., 2014) observed a lack of connectivity between the PCC and hippocampus in individuals with amnestic mild cognitive impairment who struggled with episodic memory retrieval. Dunn et al. (2014) pointed out that DMN impairment is not only a result of hippocampal atrophy but rather in combination with PCC decay.

To gain insight into the ageing process this chapter will examine a multimodal approach by investigating resting state metabolite and functional activity across age utilising ^{1}H-MRS and fMRI. In the present study we tested the hypothesis that Glu concentration levels will positively correlate and GABA+ (plus macromolecules) levels will negatively correlate with resting-state functional connectivity between the PCC and hippocampus.

6.3. Methods

6.3.1. Participants

The study was approved by Bangor University School of Psychology Ethics and Research Committee (2013-11044-A12103) and informed, written consent was obtained from all participants prior to experimental procedures. Participants were recruited through the Bangor School of Psychology participant panel, flyers, and community facilities (such as churches and supermarkets) as well as word of mouth. The inclusion criteria for the random sample included: normal to corrected-to-normal vision and hearing; fluency in English due to the nature of neuropsychological assessment; age cut-offs for younger and older adults 18 to 30 and 55 to 90 years, respectively; and MMSE total score cut-off of 23 to 30 (as reported in Chapter 5). The exclusion criteria enclosed self-reported medication usage (except contraception for females) along with history of neurological and psychiatric disorders in conjunction with MRI safety screening form criteria (as reported in Chapter 5). Nineteen healthy younger (9 females, aged 20 - 27, $M \pm SD$ = 22.32 ± 2.3 years) and 20 older (10 females, aged 56 - 84, $M \pm SD$ = 67.70 ± 7.97 years) adults took part in this study and received monetary compensation for their time. The same cohort was used in this study as in Chapter 5.

All participants were screened for cognitive decay and mood with the Mini Mental State Examination (MMSE, Folstein, Folstein & McHugh, 1975) and the Hospital Anxiety and Depression Scale (HADS) (Zigmond & Snaith, 1983), respectively. The MMSE scores for all participants (younger: $M \pm SD$ = 28.2±1.7; older: $M \pm SD$ = 28.7±1.5) were within the suggested normal range for cognition. There were no significant differences between the education levels of the two cohorts. Two participants from the younger cohort were excluded based on their scores, which fell into the moderate and severe range on the anxiety subscale. For all participants demographics and assessment mean scores are presented in Table 1. Participants (n = 5) were not excluded if their scores fall within the mild range (8-10) for anxiety, as this may have been affected by the assessment or scanning situation.

	Younger (n=19) (Mean ± SD)	Older (n=20) (Mean ± SD)	*P* values
Age	22.32 ± 2.31	67.70 ± 7.97	< .001
Gender	M 10 / F 9	M 10 / F 10	.874
Education	16.47 ± 1.22	15.65 ± 3.41	.326
HADS – Anxiety	4.79 ± 1.90	4.60 ± 2.70	.803
HADS – Depression	2.16 ± 1.86	1.85 ± 2.11	.633
MMSE	28.16 ± 1.70	28.65 ± 1.46	.339

Table 1. Presented are the demographics and neuropsychological assessment mean scores by cohort.

6.3.2. ^{1}H-MRS acquisition and analysis

T_1-weighted anatomical images (MP-RAGE; FOV = 220 x 220 x 180mm; TE = 5ms; TR = 21ms; slice thickness = 1mm; flip angle = 8°) were acquired on a 3T Philips Achieva MR scanner (Philips Health Care, Eindhoven, Netherlands) as well as ^{1}H-MRS and fMRI scans. Anatomical images were obtained with a SENSitivity-Encoded (SENSE) 32-channel head coli for placement of voxel in ROI as well as quantification purposes of CSF and tissue volumes. Please see Chapter 5 for a full description of the MRS acquisition and analysis for Glu and GABA+ concentration levels.

Briefly - Single voxel ¹H-MRS was performed using MEGA-PRESS in the PCC with a voxel size of 25 x 25 x 25mm (Figure 1). The following parameters were used for spectral acquisition: TR/TE = 2000/ 80ms; averages = 320; phase cycles = 16; spectral width = 2000 Hz; shim voxel size = 35 x 35 x 35mm). To suppress the water signal CHEmical Shift Selective (CHESS) pulses were applied. Additionally, an unsuppressed water scan was acquired of the same voxel, in order to use brain water concentration as normalization for metabolite concentrations.

All spectral analysis was performed using TARQUIN 4.3.8 (Wilson, Reynolds, Kauppinen, Arvanitis, & Peet, 2011).

CSF and tissue volume segmentation were determined using an in-house partial volume correction code to correct for partial volume effects. Transverse (T_2) relaxation values for NAA, Cr, Cho, Glu and MI were derived from Chapter's 3 and 4 findings. Literature longitudinal (T_1) relaxation values for metabolites and water were incorporated to correct for relaxation effects.
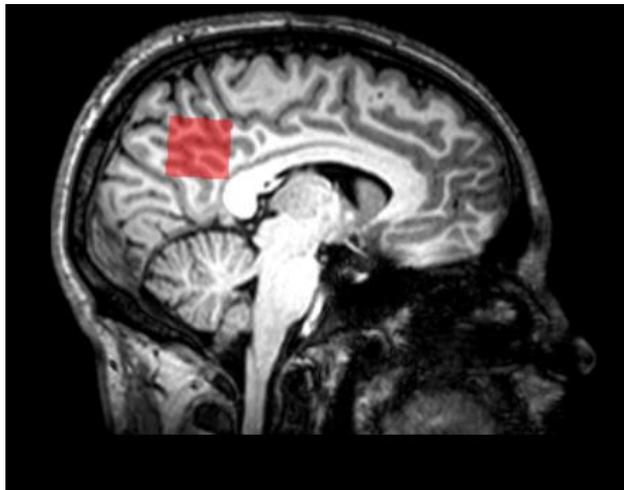
Figure 1. Anatomical image displays PCC voxel placements during ¹H-MRS acquisition in a younger participant.

6.3.3. fMRI acquisition and analysis

Resting state BOLD fMRI acquisition used gradient-echo EPI sequence with the following parameters: TR/TE = 2000/ 27ms; FOV = 240 x 240 x 131mm; flip angle = 90°; EPI factor =

39; number of slices = 37; number of dynamics = 210. During the scan participants were asked to open their eyes. Data Processing Assistant for Resting-State fMRI (DPARSF V2.2, Yan & Zang, 2010), which uses the integrated Resting-State fMRI Data Analysis Toolkit (REST V1.8, Song et al., 2011), was used for preprocessing of fMRI data. Both toolboxes use the SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/) running on MATLAB 8.3. (The MathWorks Inc., 2014). All functional images were corrected for slice timing with reference to first slice acquired. Further, motion was corrected by realignment to first volume followed by T_1-weighted anatomical image being co-registered to the mean functional image. Next, the 'New Segment' method was used to apply nuisance covariates regression to correct for six head motion parameters as well as white matter (WM), grey matter (GM) and cerebrospinal fluid (CSF) segmentation. Functional images were spatially normalized to standard Montreal Neurological Institute (MNI) template as well as smoothed by 4mm full-width-at-half-maximum Gaussian kernel and filtered to eliminate low frequency fluctuations. The seed regions, PCC [-8 -56 26, radius 5] and hippocampus [-22 -20 -26, radius 5], were chosen from Andrews-Hanna's DMN ROIs in MNI coordinate system for functional connectivity analysis (Andrews-Hanna et al., 2010b). The resulting functional connectivity correlation coefficient maps are converted by Fisher's r-to-z transformation to z maps. The DPARSF toolbox outputs the z-scores between the seed regions PCC and hippocampus, which were then used for statistical analysis.
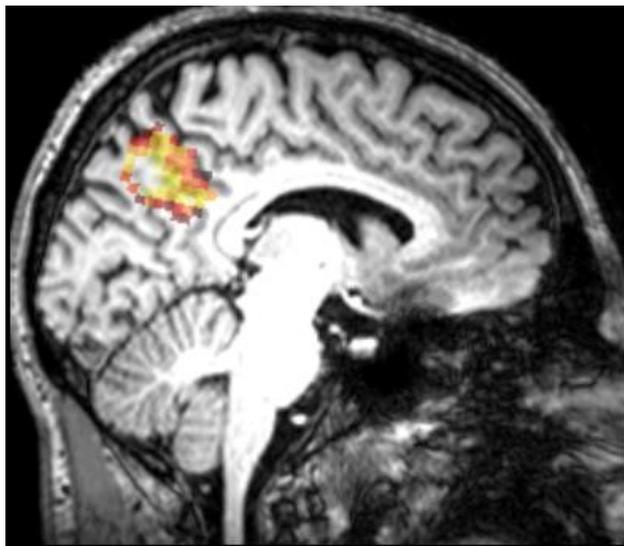
Figure 2. Example of PCC activity in the DMN of younger participant.

6.3.4. Statistical analysis

Pearson product-moment correlation coefficient (r) tests were used to determine individual Glu and GABA+ relationship to functional connectivity (FC) between PCC and hippocampus across both cohorts. To correct for multiple comparisons Bonferroni correction threshold for statistical significance was set at $p = .025$ to account for both metabolites. An independent samples t-test was used to asses any differences in FC between PCC and hippocampus between younger and older adults ($p = .05$). All statistical results are reported as two-tailed.

6.4. Results

Estimated resting-state Glu concentration ($r = .40$, $n = 34$, $p = .018$) was positively correlated with FC between PCC and hippocampus across both cohorts (Figure 3). The findings revealed no significant correlation between GABA+ and FC between PCC and hippocampus across both cohorts. In addition, we observed no significant differences between the FC of PCC and hippocampus between the younger and older adults ($p = .11$).
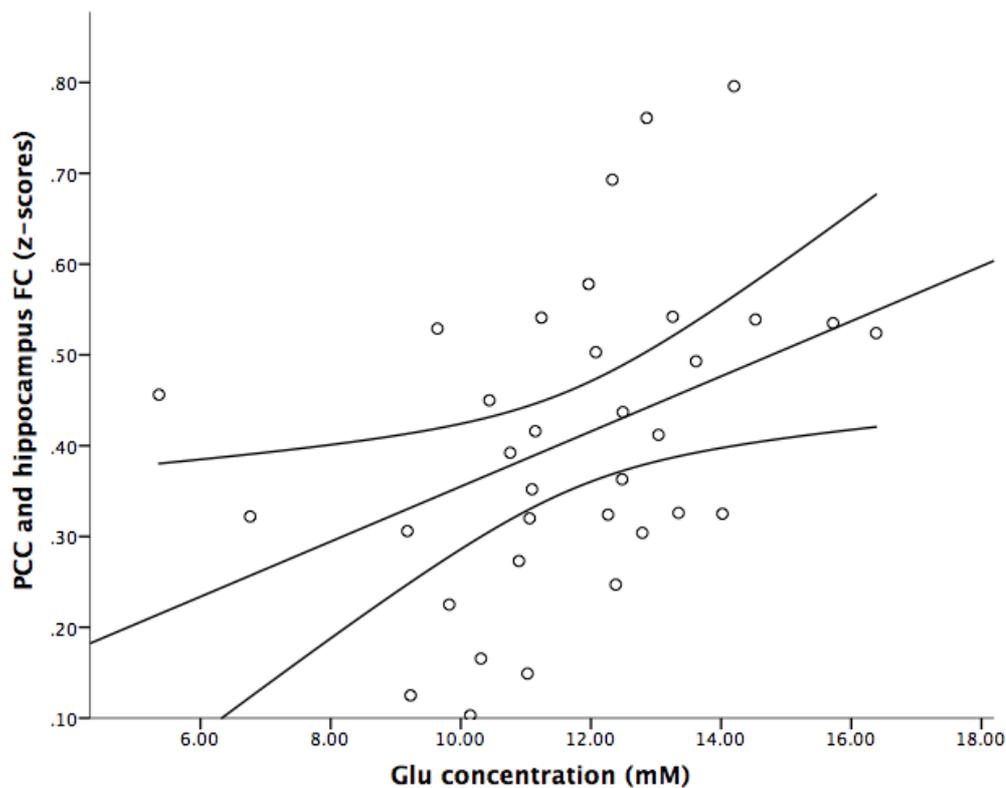
Figure 3. Scatterplot displays the relationship between Glu concentrations levels and FC between PCC and hippocampus across both cohorts.

6.5. Discussion

The current study combined ¹H-MRS and fMRI to investigate relationship between the major excitatory and inhibitory neurotransmitters and functional connectivity across age. The findings revealed a positive correlation between Glu concentration levels and FC of PCC and hippocampus. The outcome partially corresponds with our stated hypothesis. Firstly, we observed that higher resting-state Glu concentration levels were positively correlated with increased FC of PCC and hippocampus across both cohorts. However, we did not observe a significant correlation between GABA+ and FC of PCC and hippocampus across both cohorts. The research in this area is rather limited and may reflect the complexity of exploring neurophysiological mechanisms. Several studies investigating Glu concentrations with BOLD signal have explored task-based activity. Duncan et al. (2011) examined the interaction of Glu concentration with resting state related activity in a task-negative region and stimulus-induced activity in a task-positive region. The authors mentioned that Glu was a combination of glutamate/ glutamine (Glx) to creatine ratio. In our study we have separated Glu and glutamine and referenced to water, as the applied echo times of 80ms has been suggested to be optimal for minimizing glutamine and GABA contributions to the Glu peak (Mullins, Chen, Xu, Caprihan, & Gasparovic, 2008; Schubert, Gallinat, Seifert, & Rinneber, 2004). However, if we would consider the use of Glx instead, we would still observe a significant positive correlation between Glx and FC of PCC and hippocampus ($p = .017$). Duncan et al. (2011) reported a positive relationship between the perigenual anterior cingulate cortex (pgACC) Glu concentrations and the task-induced BOLD signal in the supragenual anterior cingulate cortex (sgACC). Equally a positive correlation was observed between pgACC Glx/Cr ratio and resting-state activity in the pgACC (Enzi et al., 2012). Kapogiannis et al. (2013) reported a positive Glu and negative GABA correlation with the DMN intrinsic functional connectivity. A majority of previous studies reported an inverse correlation between GABA concentration levels and BOLD signal activity (Arrubla et al., 2014; Muthukumaraswamy et al., 2012; Stagg et al., 2011). In the present study, no correlation was observed between GABA+ concentrations and FC of PCC and hippocampus. While at first this may seem at odds with the literature on GABA and it's relationship to BOLD activity and functional connectivity, a recent, rigorous study investigating correlations between task-based BOLD signal and GABA concentrations in five different brain regions (auditory cortex, frontal eye field, sensorimotor cortex, occipital cortex, and dorsolateral prefrontal cortex) (Harris et al., 2015) also reports no significant results. Taken together these results suggest the role GABA levels play in influencing BOLD activity is not as simple as others have suggested. In addition, we also found no significant differences in FC between PCC and

hippocampus between the younger and older adults. However, our result is broadly supportive of the concept of glutamatergic levels indexing some aspect of neural activity involved in functional connectivity in the human brain.

Research focuses on the balance between Glu and GABA concentrations by considering them as excitatory and inhibitory mechanisms. However, caution has to be exercised as these neurotransmitters are compartmented and a direct acquisition of this balance is not attainable with ^{1}H-MRS. Yet research acknowledges that the excitatory/inhibitory balance is neurophysiologically relevant (Kapogiannis et al., 2013). As such, some researchers regard the neurotransmitters Glu and GABA+ as representing global metabolism or proxies for excitatory and inhibitory potential. The observed association between Glu and FC does not indicate that Glu is a 'driver' of the FC between PCC and hippocampus but rather highlights the complexity of the circuitry and functional organization of the brain and the underlying mechanism (Logothetis, 2008).

The current study focused on the target regions PCC and hippocampus as a result of their involvement in memory processes (Eichenbaum, 2001). Research has demonstrated that these DMN regions are not only functionally but also structurally connected (Greicius, Supekar, Menon, & Dougherty, 2009). Several studies have observed age-related DMN changes, with the PCC as seed region (Koch et al., 2010). As well as suggesting that DMN activity can distinguish healthy ageing adults from individuals with Alzheimer's disease (Greicius et al., 2009). Therefore, future research would benefit from examining Glu and GABA concentrations in relation to FC in individuals with mild cognitive impairment and Alzheimer's disease compared to age matched groups.

6.5.1. Limitations

There are several limitations to this study that need to be considered. Firstly, the outcome has to be regarded with caution as data from four participants were excluded due to deviation from the rest of the data set. The outliers exhibited a negative correlation within the FC of PCC and hippocampus. In other words, the activity in the PCC would increase while the activity in the hippocampus would decrease. If the outliers were included in the analysis, there would be no significant correlation between Glu concentration and FC of PCC and hippocampus.

Secondly, there might be a lack of overlap between the PCC as seed region for BOLD measurement and the PCC voxel for Glu and GABA+ acquisition (Figure 2). The PCC voxel for metabolite acquisition will have contributions from GM, WM, and CSF in spite of partial volume correction.

Thirdly, as both cohorts were the same as in Chapter 5, data has to be considered with caution, as female participants were not required to provide their menstrual status. Notably, there was a difference in GABA+ concentrations between females and males in the younger age group (as mentioned in Chapter 5).

6.6. Conclusion

In summary, our data have shown that higher PCC glutamate concentrations are related to increased functional connectivity between the posterior cingulate cortex and hippocampus across age. The data is in agreement with previous research suggesting glutamatergic involvement in functional connectivity. Combining ¹H-MRS and fMRI measures of neurochemical and BOLD activation may help to explain possible differences in mechanisms underlying ageing or disease progression.

6.7. Acknowledgements

Chapter 7

General Discussion

7.1. Introduction

The current thesis aimed to enhance our understanding of the ageing process by investigating observable neurochemical alterations that may occur as we age. Specifically, transverse relaxation, metabolite concentration in relation to cognitive processes, and metabolite concentrations in relation to functional connectivity across age were investigated.

This section of the thesis contains a summary and discussion of the key findings from the systematic review and empirical studies with consideration to the research questions and existing literature. This will be followed by the consideration of the limitations and potential implications along with recommendations for future research.

The first objective of the present thesis was to establish consensus metabolite T_2 values across brain tissue content (Chapter 3). A literature review was conducted to gather studies investigating metabolite transverse relaxation in the human brain using ^{1}H-MRS.

The second objective was to determine if neurometabolite T_2 values differ between younger and older adults and how this might impact metabolite quantification with ^{1}H-MRS (Chapter 4). ^{1}H-MRS data were collected and statistically compared between healthy younger and typically ageing older adults.

The third objective was to investigate weather excitatory and inhibitory neurotransmitters predict cognitive performance across age (Chapter 5). The findings from objective 1 and 2 were incorporated in metabolite quantification acquired from a new and larger cohort. To establish any relation between neurotransmitters and cognitive performance across age, participants completed neuropsychological assessment and ^{1}H-MRS data was collected from the PCC.

The final objective was to explore the relationship between excitatory and inhibitory neurotransmitters and functional connectivity across age (Chapter 6). Building on the findings from objectives 1, 2, and 3, the relationship between neurochemical and fMRI data across age was examined. The same study cohort was used in this study as in Chapter 5.

7.2. Summary of key findings

Chapter 3 – Research question 1: What are the consensus neurometabolite T_2 values across brain tissue content?

In chapter 3, a literature search was conducted to extract studies, which examined metabolite transverse relaxation in the human brain using ^{1}H-MRS. T_2 relaxation measures provide information regarding cellular environment and pathological changes, while being a substantial part of metabolite quantification (Frahm et al., 1989; Öngür et al., 2010). Therefore, it is important to investigate potential consensus metabolite T_2 values across brain tissue content. A total of 47 studies were identified and displayed in a table format. Suggested were consensus metabolite mean T_2 values for NAA, Cr, and Cho in regions of GM, WM, and mixed tissue content at 1.5 and 3T. There was no sufficient data for other metabolites or magnetic field strength. Although different approaches were used by the included studies, this review highlights that T_2 relaxation is dependent on tissue content and has to be appropriately accounted for when quantifying metabolites.

As well as providing a collection of studies investigating T_2 relaxation times in the human brain, the review revealed a few methodological factors, such as TE, J-coupling, and exponential fit, that need to be considered when acquiring T_2 relaxation times and subsequently quantifying metabolite concentrations.

Chapter 4 – Research question 2: Do neurometabolite T_2 values differ between younger and older adults and how might this impact metabolite quantification with ^{1}H-MRS?

Chapter 4 was building on the findings of Chapter 3 by investigating metabolite T_2 relaxation times across age. The results suggested shorter mean T_2[†] relaxation times for NAA, Cho, and MI in older compared to younger adults. However, NAA was the only metabolite to survive correction for multiple comparisons. The outcome for NAA T_2[†] relaxation times corresponds with previous literature (Kirov, Fleysher, Fleysher, Patil, Liu, & Gonen, 2008; Marjańska, Emir, Deelchand, & Terpstra, 2013), however, is in contrast to one study suggesting increases in T_2 relaxation with age (Kreis, Slotboom, Hofmann, & Boesch, 2005). The finding of reduced NAA T_2[†] suggests age-related changes in the micro-environment, which NAA inhabits. This chapter also reports on the T_2 for Glutamate, a metabolite whose

T_2 relaxation is not as well characterised at 3T as other metabolites, and shows that Glutamate does not exhibit a reduction in T_2 with age. Understanding relaxation changes is especially important for studies investigating neurochemistry in ageing as reported reductions in a metabolite may actually result from a reduction in $T_2^{\dagger}$ if not properly corrected. In brief, the outcome for T_2 relaxation captures the complexity of metabolite quantification. Particularly, when investigating alterations in metabolite concentrations either in healthy ageing or pathology. These metabolite alterations may actually be $T_2^{\dagger}$ relaxations changes and misrepresent potential findings.

Chapter 5 – Research question 3: Do excitatory and inhibitory neurotransmitters predict cognitive performance across age?

Chapter 5 examined the association of neurochemical and cognitive mechanisms across age. The results suggest age and decreased PCC Glu concentration levels to be predicative of the performance on the immediate and delayed visual reproduction recall across age. In addition, age was the only predictor of performance on the Stroop interference task across both age groups. The outcome for GABA+ revealed no association to cognitive performance. As well as the mentioned findings we observed an age-related decline in Glu concentration in the PCC, which correspond with previous literature. It is not supervising that age is a large predictor in cognitive performance as previous research has reported of a decline in sensory acuity, processing speed, and spatial abilities with age (Naveh-Benjamin & Kilb, 2014; Pak, Czaja, Sharit, Rogers, & Fisk, 2008; Salthouse, 2000). We did not observe any significant findings for immediate and delayed Logical Memory. This may indicate that not all cognitive domains decline at the same time during the ageing process, as previously suggested by research. Reduced Glu concentrations have been associated with poor performance on cognitive tests as well as linked with research into signal pathways (Peng, Zhang, Zhang, Wang, & Ren, 2011; Zahr et al., 2008; Zahr et al., 2013). However, a direct link between Glu concentrations levels and cognitive performance is not possible, as the majority of Glu measured with MRS is intra-cellular and may not be directly involved in neurotransmission. Previous research has proposed Glu concentration levels to be proxies for excitatory potential (Stagg et al., 2009) or represent a measure of global metabolism in the region (Bednařík et al., 2015; Mangia et al., 2009). These findings of reduced PCC Glu concentrations may therefore reflect reductions in neuronal activity or metabolism in that region. Fonnum (1993) has suggested that decreased Glu concentrations may be due to alteration in metabolic activity.

We did not observe age-related changes in GABA+ concentration levels in the PCC. A previous study has investigated GABA(A) receptor, utilizing gene disruption, suggesting links with learning and memory in animal studies (Collinson et al., 2002). As ^{1}H-MRS does not measure differences between intra- and extracellular changes in metabolites, it is possible that subtle GABA+ alterations are not obtained.

Chapter 6 – Research question 4: What is the relationship between excitatory and inhibitory neurotransmitters and functional connectivity across age?

The study presented in Chapter 6 investigated the association of Glu and GABA concentrations in relation to functional connectivity between the PCC and hippocampus across age. The findings suggest a positive correlation between resting-state PCC Glu concentration levels and the functional connectivity between PCC and hippocampus across age. Commonly research focuses on exploring Glu levels with task-based BOLD signal activity. Several studies have reported a positive correlation between Glu concentrations with BOLD signal (Duncan et al., 2011; Enzi et al., 2012). Research investigating DMN suggested a positive correlation between Glu concentrations and DMN intrinsic functional connectivity (Kapogiannis et al., 2013). These findings are especially interesting as we reported in Chapter 5 that Glu levels are predictive of cognitive performance on selective memory tests. This association may reflect a link that glutamatergic levels have some involvement in neural activity in the human brain.

There was no significant association between GABA+ concentration and functional connectivity of PCC and hippocampus across both cohorts. Numerous studies have reported a negative correlation between GABA concentration levels and BOLD signal (Arrubla et al., 2014; Kapogiannis et al., 2013; Muthukumaraswamy et al., 2012; Stagg et al., 2011). However, a recent study investigating five different brain regions reported no relationship between task-based BOLD signal and GABA concentrations (Harris et al., 2015). The research into GABA levels effecting BOLD activity is not straight forward and requires further exploration of the complexity of these mechanisms.

7.3. Limitations

As a whole the research presented here has contributed to the literature with some interesting findings. However, some potential limitations should be considered when interpreting the results presented in this thesis.

In Chapter 4 we observed Glu $T_2^\dagger$ relaxation times to be shorter compared to the literature. This observed difference may be due to complications in measuring $T_2^\dagger$ relaxation for this metabolite, or due to the different measurement methods. One previous study by Schubert et al. (2004) highlighted that their Glu T_2 value was derived from referencing to a phantom at the same echo time. In comparison, a further study used a much longer first TE of 54ms compared to ours (Ganji et al., 2012). Here, we used a larger sample compared to the mentioned studies as well as different brain regions. In addition, some of the participants from the older cohort took medication for hypertension, thyroid dysfunction, and cholesterol. If these medical conditions are not treated, they may influence the neurochemical composition of the brain. Nevertheless, the older adults have taken their medication regularly for over 4 weeks, which is considered to be well controlled, however results should be considered with caution.

The age range for the older participants in Chapter 5 and 6 was broader compared to the younger. However, previous research has used similar age range with even smaller group sizes. As such, we feel the findings are age representative. Future research would be encouraged to investigate neurochemical changes in association to cognition according to age by decade. This may provide improved interpretation of ageing processes in relation to neurochemistry.

Furthermore, consideration has to be taken when interpreting the results from Chapter 5 and 6 due to gender effects. Our sample included both genders across age. Gender differences have previously been reported to influence Glu and Gln as well as GABA concentrations (De Bondt, De Belder, Vanhevel, Jacquemyn, & Parizel, 2015; Epperson et al., 2002). We observed gender differences in GABA+ levels in younger but not older age group. Females in the younger cohort have reduced PCC GABA+ levels in contrast to males in the same age group. As such, the findings have to be interpreted with caution.

It is also important to highlight that in Chapter 6 four outliers have been excluded from the data set, as their functional connectivity was negatively correlated between the PCC and

hippocampus. However, without the exclusion of the outliers the analysis revealed no significant results for Glu or GABA concentrations in relation to FC of PCC and hippocampus. This result has to be considered without caution and further research investigating these differences would be of benefit.

A further limitation is the placement of the spectroscopy voxel in the PCC as well as the PCC seed region for BOLD measurement might not quite overlap. Harris et al. (2015) compared the analysis of restricting a seed region to voxel of interest, while comparing it to the results of a BOLD that is not restricted to that region, and found no differences. As such, a restriction of BOLD to the spectroscopy voxel might not be necessary be effective but it would have provided a better representation of the region of interest under investigation.

7.4. Open questions and future direction

Taken together, the studies have presented new knowledge about the neurochemical mechanisms across age. However, some questions are still left open and require some consideration in the future.

There is a large interest in identifying neurochemical biomarkers in ageing and disease. The systematic review proved useful in identifying limited research on certain metabolite T_2 relaxation times. Therefore, future studies need to explore other metabolites such as Glu, GABA or MI. To acquire these metabolites, difficulties might be encountered due to their chemical environment. Yet this provides another opportunity to develop or enhance present methods for improved metabolite acquisition, which would allow investigating other factors that can have an effect on T_2 relaxation such as compartmentation or cellular structure.

In the present work we were able to link Glu concentrations with cognitive performance and BOLD activity. However, these are not direct links and further investigation is required to support these findings. Potentially, multimodal approaches incorporating blood flow measures might provide a better scope on the involvement of Glu and GABA in BOLD activity across age. Additionally, research would benefit by adopting these measures to explore alterations in mild cognitive impairment and Alzheimer's disease compared to age matched groups.

The present study in Chapter 6 investigated the brain regions PCC and hippocampus, which are part of the default mode network and have been suggested to be not only functionally but

also structurally connected (Greicius, Supekar, Menon, & Dougherty, 2009). These regions were chosen due to their involvement in memory processes. However, future research could put some emphasis by investigating other regions of the default mode network in relation to neurochemistry across age and disease.

7.5. Practical implications

The following are key points of potential practical implications drawn from the present thesis.

The systematic review has contributed by providing consensus NAA, Cr, and Cho T_2 relaxation values for improved metabolite quantification as well as revealing which metabolites require further investigations such as Glu, GABA, and MI. Building on the findings of the systematic review, we observed age-related changes in NAA T_2 relaxation values. This emphasizes the importance of appropriately correcting for T_2 relaxations, as otherwise changes in metabolite concentrations may be misrepresented and introduce errors in interpreting differences in ageing and disease. Previous research has investigated metabolite T_2 relaxation times in pathological process such as brain tumours (Isobe et al., 2002), multiple sclerosis (Sarchielli, et al.,1999), and cerebral infracts (Sappey- Marinier et al., 1992). Isobe et al. (2002) reported shorter NAA and Cr T_2 relaxation times and increased Cho T_2 relaxation time in individuals with gliomas and healthy controls. A study investigating metabolite T_2 relaxation time differences between individuals with multiple sclerosis and healthy controls found no significant T_2 relaxation times differences for NAA, Cr, or Cho (Sarchielli, et al.,1999). While Cr T_2 relaxation time has been observed to be increased in cerebral infracts compared to normal white matter, resulting in higher Cr concentration levels in individuals with chronic cerebral infarcts/ stroke compared the healthy controls (Sappey-Marinier et al., 1992). Caution should be taken when interpreting these outcomes, as unknown factors such as intracellular energy metabolism may influence the results. Metabolite T_2 relaxation measures have been applied in mood disorders such as bipolar disorder and schizophrenia to provide biological information. Essentially, T_2 relaxation may help and support in understanding the processes during ageing and disease. It would be of interest to broaden the investigation into metabolite T_2 relaxation times to other conditions such as long-term depression versus long-term potentiation.

The practical implications for neurochemical links to cognition and functional connectivity are complex. Glu concentrations have been linked to both cognitive performance and functional connectivity between PCC and hippocampus across age. However, these are not direct links

and have to be considered with caution. Chapter 5 and 6 have highlighted the complexity of neurophysiological mechanisms and the measurement of them. Nevertheless, the findings that have been obtained from both studies link Glu concentrations to cognitive performance and functional connectivity across age.

7.6. Final remark

Overall, the thesis presents interesting findings along with new contributions in regard to brain chemistry in relation to cognitive performance and functional connectivity across age. These findings have practical implication and highlight shortcomings in the literature, which can be incorporated in future research. The utilization of neuroimaging techniques such as proton magnetic resonance spectroscopy as well as functional magnetic resonance imaging can provide underpinnings of healthy ageing and pathologies.

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Appendices

Appendix A

Subject: FW: Ethical approval granted for 2012-6522-A13630 Amendment to to On going technique development and quality testing of Philips 3.0 Tesla MRI system

Date: Thursday, 21 April 2016 15:36:57 British Summer Time

From: Paul Mullins

To: Karolina Rusiak

On 08/12/2015, 11:29, "ethics@bangor.ac.uk" <ethics@bangor.ac.uk> wrote:

> Dear Paul,
>
> 2012-6522-A13630 Amendment to to On going technique development and quality testing of Philips 3.0 Tesla MRI system
>
> Your research proposal number 2012-6522-A13630
> has been reviewed by the Psychology Ethics and Research Committee
> and the committee are now able to confirm ethical and governance approval for the above research on the basis described in the application form, protocol and supporting documentation. This approval lasts for a maximum of three years from this date.
>
>
> Ethical approval is granted for the study as it was explicitly described in the application
>
> If you wish to make any non-trivial modifications to the research project, please submit an amendment form to the committee, and copies of any of the original documents reviewed which have been altered as a result of the amendment. Please also inform the committee immediately if participants experience any unanticipated harm as a result of taking part in your research, or if any adverse reactions are reported in subsequent literature using the same technique elsewhere.

Rhif Elusen Gofrestredig 1141565 - Registered Charity No. 1141565

Mae'r e-bost yma'n amodol ar delerau ac amodau ymwadiad e-bost Prifysgol Bangor. Gellir darllen testun llawn yr ymwadiad yma.<http://www.bangor.ac.uk/emaildisclaimer>

This email is subject to the terms and conditions of the Bangor University email disclaimer. The full text of the disclaimer can be read here<http://www.bangor.ac.uk/emaildisclaimer>

Appendix B

Subject: Ethics Application Approved

Date: Monday, 6 October 2014 08:51:58 British Summer Time

From: Bangor Research Applications

To: Karolina Rusiak

Dear Karolina,

2013-11044-A12103 Amendment to The relationship between brain metabolites and default mode network in normal ageing: A 1H-MRS and fMRI study.

Your research proposal number 2013-11044-A12103 has been reviewed by the School of Psychology Ethics and Research Committee and the committee are now able to confirm ethical and governance approval for the above research on the basis described in the application form, protocol and supporting documentation. This approval lasts for a maximum of three years from this date.

Ethical approval is granted for the study as it was explicitly described in the application

If you wish to make any non-trivial modifications to the research project, please submit an amendment form to the committee, and copies of any of the original documents reviewed which have been altered as a result of the amendment. Please also inform the committee immediately if participants experience any unanticipated harm as a result of taking part in your research, or if any adverse reactions are reported in subsequent literature using the same technique elsewhere.
.

Governance approval is granted for the study as it was explicitly described in the application and we are happy to confirm that this study is now covered by the University's indemnity policy.

If any new researchers join the study, or any changes are made to the way the study is funded, or changes that alter the risks associated with the study, then please submit an amendment form to the committee.

Yours sincerely

Everil McQuarrie

--
Rhif Elusen Gofrestredig / Registered Charity No. 1141565

Mae'r e-bost yma'n amodol ar delerau ac amodau ymwadiad e-bost Prifysgol
Bangor. Gellir darllen testun llawn yr ymwadiad yma:
http://www.bangor.ac.uk/emaildisclaimer
This email is subject to the terms and conditions of the Bangor University
email disclaimer. The full text of the disclaimer can be read here:
http://www.bangor.ac.uk/emaildisclaimer

Appendix C

SONA experiment

Title: The relationship between brain metabolites and default mode network in normal ageing: A ^{1}H-MRS and fMRI study.

PI: Ms Karolina Rusiak and Dr Paul Mullins

Neuroimaging Research Study: This study is designed to test cognition and acquire images of brain structure, function and chemistry with new MRI techniques. Magnetic Resonance Imaging is non-invasive, including no radiation or injections. We hope to better understand chemistry and function in the brain across different ages.

What will you be asked to do?
There are two parts to this study. Firstly, you will be asked to complete a series of tasks (such as questionnaires) that require cognitive skills. The second part will involve you lying still in a scanner while images are obtained from your brain. The whole study will take around 2.5 hours, including breaks.

Preferred criteria (eligible)?
- Age: 18 – 30
- Free of memory complains
- Fluent English
- Stability of permitted medications for 4 weeks
- No presence of pacemakers, shrapnel, or other metal implants/objects in the eyes, skin, or body which cannot be removed prior to the scan
- Not Claustrophobic
- You don't have cerebrovascular disease, ischaemic heart disease, depression, psychiatric diseases, or history of stroke or seizure
- [No alcohol and drug abuse the previous night before testing]

What are the benefits?
You will have made a contribution to our understanding of the relationship between brain and behaviour. However, there are no direct benefits to you for your participation in the study.

Compensation: course credit = 4   printer credit = £10

Volunteers
wanted for Research Study

Neuroimaging Research Study:
This study is designed to test cognition and acquire images of brain structure, function and chemistry with new MRI techniques. Magnetic Resonance Imaging is noninvasive, including no radiation or injections. We hope to better understand chemistry and function in the brain across different ages.

What will you be asked to do?
There are two parts to this study. Firstly, you will be asked to complete a series of tasks (such as questionnaires) that require cognitive skills. The second part will involve you lying still in a scanner while images are obtained of your brain.

What are the benefits?
You will have made a contribution to our understanding of the relationship between brain and behavior. However, there are no direct benefits to you for your participation in the study.

If you have any questions, wish to receive more information regarding the eligibility, or are interested in participating, please contact: Karolina Rusiak at 01248 388569 or email: k.rusiak@bangor.ac.uk

This research study has been approved by the School of Psychology Research Ethics and Governance Committee (2013-11044) on the 25th November 2013.

Who is eligible?

- 55 years old and over
- no metal in eyes, skin, or body, including pacemakers or implants
- not claustrophobic
- no history of stroke or seizure

Compensation

The experiment will last around 2.5 hours, including breaks. To thank you for your time we will compensate you with £20 and refreshments will be provided. The study will be in School of Psychology, Bangor University.

Neuroimaging research study
Phone: 01248 388569 Karolina
Email: k.rusiak@bangor.ac.uk

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Volunteers
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Neuroimaging Research Study:
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What will you be asked to do?
There are two parts to this study. Firstly, you will be asked to complete a series of tasks (such as questionnaires) that require cognitive skills. The second part will involve you lying still in a scanner while images are obtained of your brain.

What are the benefits?
You will have made a contribution to our understanding of the relationship between brain and behavior. However, there are no direct benefits to you for your participation in the study.

If you have any questions, wish to receive more information regarding the eligibility, or are interested in participating, please contact:
Karolina Rusiak at 01248 388569 or Email: k.rusiak@bangor.ac.uk

This research study has been approved by the School of Psychology Research Ethics and Governance Committee (2013-11044).

Who is eligible?

- **Age: 18 - 30**
- **no metal in eyes, skin, or body, including pacemakers or implants**
- **not claustrophobic**
- **no history of stroke or seizure**

Compensation

The experiment will last around 2.5 hours, including breaks. To thank you for your time we will compensate you with £20 and refreshments will be provided. The study will be in School of Psychology, Bangor University.

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Appendix F

PRIFYSGOL
BANGOR
UNIVERSITY

BANGOR BRAIN IMAGING UNIT
Participant Information Sheet

School of Psychology: Bangor University
Information Sheet for Participating in a Research Project

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

TITLE OF STUDY:
"The relationship between brain metabolites and default mode network in normal ageing: A 1H-MRS and fMRI study."

INVESTIGATORS:
Miss Karolina Rusiak (PhD student/ Research Support Officer)
Dr Paul Mullins (Project supervisor)

WHAT IS THE PURPOSE OF THE STUDY?
This study is designed to test cognition and acquire images of brain structure, function and neurochemistry with new MRI techniques. We hope to better understand neurochemistry and function in the brain across different ages. In addition, we would like to better understand cognitive performance with brain neurochemistry levels across age.

WHAT ARE THE PROCEDURES?
Cognitive testing: In this part of the research study you will be asked to complete a series of tasks that require cognitive skills. This session will last between 1 – 1.5 hours. You will have the opportunity to take some breaks if you feel tired.
To be able to take part in this part of research you will have the following:
- visual and auditory acuity adequate for testing
- fluent in English

MRI Scanning: The study involves lying still in the scanner while images are obtained. The MRI scanner uses a magnetic field – no radiation is involved and no dye needs to be injected. The scan is not in any way painful, but the scanner makes a loud noise so we will give you ear plugs as well as headphones to reduce this noise.

You will be able to see outside the scanner during the scan and will be able to communicate with the operator. If you find the scan to be uncomfortable in any way, the operator will immediately stop the scan.

This study will include MR measurements of static brain anatomy, function, and brain chemistry; these require nothing on your part except that you remain still in the scanner.

Because a magnetic field is involved, you cannot be scanned if you have the following:
- a pacemaker, or other metal implants/ objects in your eyes, skin, or body, which cannot be removed prior to the scan
- claustrophobic (fear of confined spaces)
- history of stroke or seizure
- pregnant
We will go through a list of relevant items with you before scanning.

The scanning session will take about 1- 1.5 hours, although you will not actually be scanned for more than 1 hour of this time.

At the end of the scanning session you will be debriefed and will have an opportunity to ask questions.

WHAT IS THE DEVICE INVOLVED?

We can learn a great deal about how the brain works by looking at the blood flow to, and chemistry of, different parts of the brain whilst at rest and while performing different tasks. We need to obtain this information in both health and disease.

We measure brain function using images taken with a magnetic resonance imaging scanner. This scanner uses a strong magnetic field to create detailed images of brain structure and function. By taking a series of images whilst you lay still we can build up a picture of the brain areas activated by this type of function. The scan does not involve any injections or X-rays.

ARE THERE ANY RISKS?

The scanner can be loud when it takes images, and you will be given earplugs and ear defenders to block out some of the sound. Also, the MR environment is quite confined, and people who are uncomfortable in small or confined spaces may not be able to participate. If this should be you, remember that you may withdraw from the study at any time without explaining why.

Otherwise, given that the procedure involves a non-invasive imaging technique it is not painful or dangerous in any way. There are no known risks or side effects.

WHAT ARE THE BENEFITS?

You will have made a contribution to our understanding of the relationship between brain and behavior. However, there are no direct benefits to you of your participation in the study.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

If the new information pertains specifically to the health of the volunteer, the volunteer will be informed. Otherwise, new information will be disseminated through traditional scientific channels (e.g. journal articles, conference presentations).

HOW IS CONFIDENTIALITY ENSURED?

The information obtained from the assessments may be published in scientific journals, but your name will not appear in any public document, nor will the results be published in a form, which would make it possible for you to be identified.

WHO WILL HAVE ACCESS TO THE DATA?

Members of the BANGOR BRAIN IMAGING UNIT will have access to the data. It is possible that the data may be used by researchers working with the BANGOR BRAIN IMAGING UNIT for other similar ethically approved research protocols, where the same standards of confidentiality will apply. The BANGOR BRAIN IMAGING UNIT complies with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing, and disclosure of personal information. All enquiries concerning access to the data held by the BANGOR BRAIN IMAGING UNIT should be addressed to the Freedom of Information Liaison Officer at the Unit in the first instance.

DO I HAVE A RIGHT TO REFUSE OR WITHDRAW?

You may refuse to participate at any time. You may change your mind about being in the study and quit after the study has started, and if you feel, for any reason, uncomfortable, the study will be discontinued.

WILL MY GP BE INFORMED?

Your GP will not be routinely informed if your participation in this study has been as a normal volunteer.

What if there is something wrong with my brain, would it show up on my images?

This is an important question, and one that can't be answered with a straight yes or no answer. The information below hopes to provide an answer. If you still have questions, please ask the researcher for more information.

There is the potential that an unexpected abnormality will be found in your scan. The likely hood of such an abnormality identifiable in a normal volunteer's scan is estimated to be between 2-10%, so you should be aware that such a possibility exists.

The MRI scans being done as part of the study you are participating in are **designed to answer research questions and not to provide a medical diagnosis**. They may not show problems that a ordinary clinical scan

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would, and since the scientists reviewing the scans are generally not medical doctors, they may fail to notice such abnormalities.

However if something out of the ordinary is suspected in one of your scans, we will ask a neurologist, who is a medical doctor with experience interpreting brain MRI scans and treating brain disorders, to review the images with us. The neurologist will not be told your name, although they may be told your age and gender. If they think there may be a problem, we will then contact you. You will be offered the opportunity to meet and have a discussion with the neurologist about the findings and your options.

If you have a GP and you agree, we will contact her/him and pass the scans along with the recommendation from the neurologist. We will only contact your GP with your permission and if your brain scans show something of potential medical concern. These scans do not routinely become a part of a medical record, however, if a problem is detected and with your permission the images are sent to a medic involved in caring for you, they may become part of your medical record. There is also the possibility that you may be unduly worried if a problem is suspected, but is not actually found.

If in the future symptoms do arise, do not assume that because your brain has been scanned and we haven't contacted you that there is not a problem. Please take any future concerns to your GP, we can make the images available if required.

WHAT WILL HAPPEN TO THE STUDY RESULTS?
They will be kept securely for a minimum of 10 years and possibly indefinitely in the BANGOR BRAIN IMAGING UNIT data archive in accordance with good research practice. Results of the study may be published in a scientific journal or other public format. In this case your data will either be included as part of a group average, or will be anonymised so that no identifying information is given.

WHAT IF I HAVE FURTHER QUESTIONS?
We welcome the opportunity to answer any question you may have about any aspect of this study or your participation in it. Please contact Paul Mullins at the School of Psychology, University of Wales, Bangor, Gwynedd, LL57 2AS, phone 01248 383631.

ARE THERE COMPENSATION ARRANGEMENTS IF SOMETHING GOES WRONG?
In the unlikely event of anything untoward happening, the University's insurer provides insurance for negligent harm. It does not provide insurance for non-negligent harm but does take a sympathetic view should a claim be made.

WHAT IF I HAVE COMPLAINTS?
This research study has been approved by the School of Psychology Research Ethics and Governance Committee. In the case of any complaints concerning the conduct of research, please address these to Mr Hefin Francis, School Manager, School of Psychology, Bangor University, Gwynedd, LL57 2AS.

Thank you for considering taking part in this study. Our research depends entirely on the goodwill of potential volunteers such as you. If you require further information, we will be pleased to help you in any way we can.

BANGOR BRAIN IMAGING UNIT
Participant Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF STUDY: "The relationship between brain metabolites and default mode network in normal ageing: A ^{1}H-MRS and fMRI study."

INVESTIGATORS:
Dr Paul Mullins (Project supervisor)
Miss Karolina Rusiak (PhD student/ Research Support Officer)

The volunteer should complete this entire sheet himself/herself.
Please circle as appropriate:

Have you read the participant information sheet?

YES / NO

Have you had the opportunity to ask questions and discuss this study?

YES / NO

Have you received enough information about the study?

YES / NO

Do you understand that your participation is voluntary and that you are free to withdraw from the study:

    - At any time
    - Without having to give a reason
    - And without affecting your future medical care?

YES / NO

Do you understand that this is not a diagnostic scan, but that should something abnormal be noticed, this finding will be discussed with you?

YES / NO

Do you understand that the Bangor University provides insurance for negligent harm but that it does not provide insurance for non-negligent harm?

YES / NO

No. _____

Do you understand that the research data may be accessed by researchers working at or in collaboration with the BANGOR BRAIN IMAGING UNIT in similar ethically approved studies, but that at all times your personal data will be kept confidential in accordance with data protection guidelines?

YES / NO

Do you agree to take part in this study?

YES / NO

We may in future wish to ask you to take part in similar studies, is it okay for us to contact you to ask if you are interested in doing so? (your answer here has no bearing on your current participation in this study)

YES / NO

_____ _____
Date   Signature of
                                                                           Participant
                 _____
                                                                     Name in block letters

_____ _____
Date   Signature of
                                                                          Investigator
                 _____
                                                                     Name in block letters

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Study Number: _____ PI: _____

BANGOR BRAIN IMAGING UNIT
MR Safety Screening Questionnaire

PRIFYSGOL
BANGOR
UNIVERSITY

Name	BANGOR BRAIN IMAGING UNIT no. (Staff Use Only)
Phone number	Date of Birth
Email address	Weight (kg)

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the Scanner Room with any metal in or on your body or clothing.

Please answer the following questions carefully and ask if anything is not clear.

All information is held in the strictest confidence.

Circle one answer for each question.

1. Do you have a pacemaker or artificial heart valve?   Y/N
2. Do you have aneurysm clips (clips put around blood vessels during surgery)?   Y/N
3. Do you have any implants in your body? (e.g., replacement joints, drug pumps, metal pins, plates, coronary stents, breast implants etc).   Y/N
4. Have you ever had any metal fragments in your eyes?   Y/N
5. Have you ever worked with metal (e.g., grinding, machining, welding) without eye protection?   Y/N
6. Do you have any metal or shrapnel fragments anywhere in your body?   Y/N
7. Do have an indwelling catheter in your body?   Y/N
8. Have you ever had an operation on your head, spine, or chest?   Y/N
9. Have you ever had any other surgery (if yes, please give brief details)?   Y/N
        Details _____
10. Do you have any implanted electrical devices (e.g., hearing aid, cochlea implant, nerve stimulator)?   Y/N
11. Have you ever had an EEG or brain scan?   Y/N
12. Have you ever had an MRI scan before?   Y/N
13. Do you wear dentures, a dental plate, or a brace (not fillings)?   Y/N
14. Do you have any transdermal patches? (skin patches)   Y/N
15. Do you have any tattoos or body piercings?   Y/N
16. Is there any possibility that you could be pregnant?   Y/N
17. Are you susceptible to claustrophobia?   Y/N
18. Do you have hypertension (high blood pressure) sufficient to require medication?   Y/N
19. If Yes to 18 above, has your hypertension been adequately treated by medication?   Y/N
20. Have you had or do you have any heart problems?   Y/N
21. Do you have an impaired ability to perspire?   Y/N

Version 01 07/02/2014

PRIFYSGOL
BANGOR
UNIVERSITY

BANGOR BRAIN IMAGING UNIT
MR Safety Screening Questionnaire

Name	BANGOR BRAIN IMAGING UNIT no. (Staff Use Only)
Phone number	Date of Birth
Email address	Weight (kg)

MR scanning uses strong magnetic fields. For your own safety and the safety of others it is **very important** that you do not go into the Scanner Room with any metal in or on your body or clothing.

Please answer the following questions carefully and ask if anything is not clear.

All information is held in the strictest confidence.

Circle one answer for each question.

1. Do you have a pacemaker or artificial heart valve?   Y/N
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3. Do you have any implants in your body? (e.g., replacement joints, drug pumps, metal pins, plates, coronary stents, breast implants etc).   Y/N
4. Have you ever had any metal fragments in your eyes?   Y/N
5. Have you ever worked with metal (e.g., grinding, machining, welding) without eye protection?   Y/N
6. Do you have any metal or shrapnel fragments anywhere in your body?   Y/N
7. Do have an indwelling catheter in your body?   Y/N
8. Have you ever had an operation on your head, spine, or chest?   Y/N
9. Have you ever had any other surgery (if yes, please give brief details)?   Y/N
       Details _____
10. Do you have any implanted electrical devices (e.g., hearing aid, cochlea implant, nerve stimulator)?   Y/N
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16. Is there any possibility that you could be pregnant?   Y/N
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19. If Yes to 18 above, has your hypertension been adequately treated by medication?   Y/N
20. Have you had or do you have any heart problems?   Y/N
21. Do you have an impaired ability to perspire?   Y/N

Version 01 07/02/2014

Appendix G

BANGOR BRAIN IMAGING UNIT
Participant Information Sheet

School of Psychology: Bangor University
Information Sheet for Participating in a Research Project

You are being invited to take part in a research study. Before you decide whether to participate, it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part.

TITLE OF STUDY:
"On going Image and Spectroscopy Technique Development and Quality Testing of Philips 3.0 Tesla MRI system"

INVESTIGATORS:
The study is organised by Paul Mullins, Paul Downing, Alison Wiggett, Karolina Rusiak, Emily Cross, Richard Ramsey, Patricia Bestelmeyer, Ayelet Sapir, Giovanni D'avosa and Robert Rafal from the BANGOR BRAIN IMAGING UNIT, School of Psychology, University of Wales Bangor.

WHAT IS THE PURPOSE OF THE STUDY?
Techniques to produce Magnetic Resonance Images (MRI) are constantly evolving to produce better MRI in shorter times. This study is designed to test and evaluate the usefulness of some of these new MRI techniques in producing good quality functional (fMRI), anatomical and chemical (MRS) images of brain structure, function and neurochemistry here at Bangor University. New scanning protocols to be tested will be based on techniques used at other 3T MRI sites, and compared with current standard techniques in order to have a common benchmark for expected results.

WHAT ARE THE PROCEDURES?
The study involves lying still in the scanner while images are obtained. The MRI scanner uses a magnetic field – no radiation is involved and no dye needs to be injected. The scan is not in any way painful, but the scanner makes a loud noise so we will give you ear plugs as well as headphones to reduce this noise.

You will be able to see outside the scanner during the scan and will be able to communicate with the operator. If you find the scan to be uncomfortable in any way, the operator will immediately stop the scan.

This study will include MR measurements of static brain anatomy; these require nothing on your part except that you remain still in the scanner. The remainder of the session may comprise studies of brain chemistry, or tests of brain activity in simple visual, auditory, or motor tasks. In these tests you may be given simple instructions (e.g. press a key when you see the same image twice) or you may simply be asked to pay attention to the stimuli. For visual tasks this may include: watching rotating flickering wedges, oscillating high-contrast rings, or photographs of common objects and of people. For auditory cortex this may include spoken material presented over headphones, environmental sounds, or pure tones. For motor tasks, this may include instructions to alternately clench/unclench the left and right hands, move your feet, or to manipulate an MRI-compatible "robot arm".

Because a magnetic field is involved, you cannot be scanned if you have a pacemaker, or metal in your body. We will go through a list of relevant items with you before scanning. Because the scanner is configured as a narrow tube, some individuals with claustrophobia (fear of confined spaces) may find the procedure uncomfortable or intolerable. So, you cannot be scanned if you have a history of claustrophobia.

The scanning session will take about 1-2 hours, although you will not actually be scanned for more than 1.5 hours of this time.

WHAT IS THE DEVICE INVOLVED?

We can learn a great deal about how the brain works by looking at the blood flow to, and chemistry of, different parts of the brain whilst at rest and while performing different tasks. We need to obtain this information in both health and disease.

We measure brain function using images taken with a magnetic resonance imaging scanner. This scanner uses a strong magnetic field to create detailed images of brain structure and function. By taking a series of images whilst you perform a task we can build up a picture of the brain areas activated by this type of function. The scan does not involve any injections or X-rays.

ARE THERE ANY RISKS?

The scanner can be loud when it takes images, and you will be given earplugs and ear defenders to block out some of the sound. Also, the MR environment is quite confined, and people who are uncomfortable in small or confined spaces may not be able to participate. If this should be you, remember that you may withdraw from the study at any time without explaining why.

Otherwise, given that the procedure involves a non-invasive imaging technique it is not painful or dangerous in any way. There are no known risks or side effects.

WHAT ARE THE BENEFITS?

You will have made a contribution to our understanding of the relationship between brain and behavior. However, there are no direct benefits to you of your participation in the study.

WHAT IF NEW INFORMATION BECOMES AVAILABLE?

If the new information pertains specifically to the health of the volunteer, the volunteer will be informed. Otherwise, new information will be disseminated through traditional scientific channels (e.g. journal articles, conference presentations).

HOW IS CONFIDENTIALITY ENSURED?

The information obtained from the assessments may be published in scientific journals, but your name will not appear in any public document, nor will the results be published in a form which would make it possible for you to be identified.

WHO WILL HAVE ACCESS TO THE DATA?

Members of the BANGOR BRAIN IMAGING UNIT will have access to the data. It is possible that the data may be used by researchers working with the BANGOR BRAIN IMAGING UNIT for other similar ethically approved research protocols, where the same standards of confidentiality will apply. The BANGOR BRAIN IMAGING UNIT complies with the requirements of the Data Protection Act 1998 with regard to the collection, storage, processing, and disclosure of personal information. All enquiries concerning access to the data held by the BANGOR BRAIN IMAGING UNIT should be addressed to the Freedom of Information Liaison Officer at the Unit in the first instance.

DO I HAVE A RIGHT TO REFUSE OR WITHDRAW?

You may refuse to participate at any time. You may change your mind about being in the study and quit after the study has started, and if you feel, for any reason, uncomfortable, the study will be discontinued.

WILL MY GP BE INFORMED?

Your GP will not be routinely informed if your participation in this study has been as a normal volunteer.

What if there is something wrong with my brain, would it show up on my images?

This is an important question, and one that can't be answered with a straight yes or no answer. The information below hopes to provide an answer. If you still have questions, please ask the researcher for more information.

There is the potential that an unexpected abnormality will be found in your scan. The likely hood of such an abnormality identifiable in a normal volunteer's scan is estimated to be between 2-10%, so you should be aware that such a possibility exists.

The MRI scans being done as part of the study you are participating in are designed to answer research questions and not to provide a medical diagnosis. They may not show problems that a ordinary clinical scan would, and since the scientists reviewing the scans are generally not medical doctors, they may fail to notice such abnormalities.

However if something out of the ordinary is suspected in one of your scans, we will ask a neurologist, who is a medical doctor with experience interpreting brain MRI scans and treating brain disorders, to review the images with us. The neurologist will not be told your name, although they may be told your age and gender. If they think there may be a problem, we will then contact you. You will be offered the opportunity to meet and have a discussion with the neurologist about the findings and your options.

If you have a GP and you agree, we will contact her/him and pass the scans along with the recommendation from the neurologist. We will only contact your GP with your permission and if your brain scans show something of potential medical concern. These scans do not routinely become a part of a medical record, however, if a problem is detected and with your permission the images are sent to a medic involved in caring for you, they may become part of your medical record. There is also the possibility that you may be unduly worried if a problem is suspected, but is not actually found.

If in the future symptoms do arise, do not assume that because your brain has been scanned and we haven't contacted you that there is not a problem. Please take any future concerns to your GP, we can make the images available if required.

WHAT WILL HAPPEN TO THE STUDY RESULTS?
They will be kept securely for a minimum of 10 years and possibly indefinitely in the BANGOR BRAIN IMAGING UNIT data archive in accordance with good research practice. Results of the study may be published in a scientific journal or other public format. In this case your data will either be included as part of a group average, or will be anonymised so that no identifying information is given.

WHAT IF I HAVE FURTHER QUESTIONS?
We welcome the opportunity to answer any question you may have about any aspect of this study or your participation in it. Please contact Paul Mullins at the School of Psychology, University of Wales, Bangor, Gwynedd, LL57 2AS, phone 01248 383631.

ARE THERE COMPENSATION ARRANGEMENTS IF SOMETHING GOES WRONG?
In the unlikely event of anything untoward happening, the University's insurer provides insurance for negligent harm. It does not provide insurance for non-negligent harm but does take a sympathetic view should a claim be made.

WHAT IF I HAVE COMPLAINTS?
This research study has been approved by the School of Psychology Research Ethics and Governance Committee. In the case of any complaints concerning the conduct of research, please address these to Hefin Francis, School Manager, School of Psychology, Bangor University, Gwynedd, LL57 2AS.

Thank you for considering taking part in this study. Our research depends entirely on the goodwill of potential volunteers such as you. If you require further information, we will be pleased to help you in any way we can.

BANGOR BRAIN IMAGING UNIT
Participant Consent Form

CONSENT TO PARTICIPATE IN A RESEARCH STUDY

TITLE OF STUDY: "On going Image and Spectroscopy Technique Development and Quality Testing of Philips 3.0 Tesla MRI system"

INVESTIGATORS:
The study is organised by Paul Mullins, Paul Downing, Alison Wiggett, Karolina Rusiak, Emily Cross, Richard Ramsey, Patricia Bestelmeyer, Ayelet Sapir, Giovanni D'avosa and Robert Rafal from the BANGOR BRAIN IMAGING UNIT, School of Psychology, University of Wales Bangor.

The volunteer should complete this entire sheet himself/herself.
Please circle as appropriate:

Have you read the participant information sheet?

YES / NO

Have you had the opportunity to ask questions and discuss this study?

YES / NO

Have you received enough information about the study?

YES / NO

Do you understand that your participation is voluntary and that you are free to withdraw from the study:

- At any time
- Without having to give a reason
- And without affecting your future medical care?

YES / NO

Do you understand that this is not a diagnostic scan, but that should something abnormal be noticed, this finding will be discussed with you?

YES/NO

Do you understand that the Bangor University provides insurance for negligent harm but that it does not provide insurance for non-negligent harm?

YES/NO

Do you understand that the research data may be accessed by researchers working at or in collaboration with the BANGOR BRAIN IMAGING UNIT in similar ethically approved studies, but that at all times your personal data will be kept confidential in accordance with data protection guidelines?

YES/NO

Do you agree to take part in this study?

YES / NO

We may in future wish to ask you to take part in similar studies, is it okay for us to contact you to ask if you are interested in doing so? (your answer here has no bearing on your current participation in this study)

YES / NO

_____   _____
      Date   Signature of
Participant

                    _____
                                                            Name in block letters

_____   _____
      Date   Signature of
Investigator

                    _____
                                                            Name in block letters

Appendix H

Debriefing Form for Participants
School of Psychology: Bangor University

Study Title: The relationship between brain metabolites and default mode network in normal ageing: A ^{1}H-MRS and fMRI study.

The purpose of this study was to find out if neurochemical changes paly a role in the functional connectivity between brain areas in different age groups, by using cognitive tasks and scanning sessions.

How was this tested? In this study, you were asked to perform some tasks, which tested your cognitive skills, such as memory and executive functions. Executive functions is a set of mental process that help with organizing, planning, remembering, and much more. This was followed by a scan where you were asked to lay still. The obtained data allows us to explore cognitive performance in relation to brain chemistry and connectivity between brain areas.

The information obtained from the assessments and scans may be published in scientific journals, but your name will not appear in any public document, nor will the results be published in a form, which would make it possible for you to be identified.

Hypotheses and main questions:
We expect to find that brain chemistry will change according to the strength of connectivity between brain areas. In addition, we expect the brain chemistry levels to predict performance on the cognitive tasks.

It is difficult to answer these types of questions, and your generosity and willingness to participate in this study are greatly appreciated. Your input will help contribute to the advancement of the field of ageing and dementia.

Why is this important to study? Dementia is a rising condition in the elderly population of the twentieth century. The Alzheimer's Society has estimated that around 800,000 people were diagnosed with dementia in the UK in 2012, however, this number only represents 41% of people who actually receive a diagnosis with dementia. Dementia is characterized by an on-going deterioration of cerebral structure causing a progressive decline of cognitive functions, such as memory, and decline in functional ability, such as performing everyday activities. Of particular interest to the study of dementia, research has suggested that the default mode network may be coupled with the memory system. This network is a substantial structure, which is characterized by reduced metabolism, increased deposition of amyloid, and degeneration in Alzheimer's disease. The posterior cingulate cortex (PCC), a key part of the default mode network and activated during episodic memory retrieval, shows disrupted connectivity in neuroimaging studies. Therefore, we are interested in the PCC's chemical set up and connectivity combined with memory performance across age.

What if I want to know more?

If you are interested in learning more about ***this area of research***, you may wish to consult:

- www.neuroskill.eu or www.alzheimers.org.uk or www.nhs.uk/Conditions/dementia-guide/Pages/ about-dementia.aspx
- Tartaglia, M., Rosen, H., & Miller, B. (2011). Neuroimaging in Dementia. *Neurotherapeutics*, 8(*1*), 82-92.
- Reyngoudt, H., Claeys, T., Vlerick, L., et al. (2012). Age-related differences in metabolites in the posterior cingulate cortex and hippocampus of normal ageing brain: a 1H-MRS study. *European journal of radiology*, 81 (*3*), 223-31.

If you are interested in learning more about ***memory***, you may wish to consult

- NHS website   http://www.nhs.uk/conditions/memory-loss/Pages/Introduction.aspx
- Help guide website   http://www.helpguide.org/life/prevent_memory_loss.htm

If you are interested in learning more about ***depression***, you may wish to consult:

- NHS website   http://www.nhs.uk/conditions/depression/pages/introduction.aspx
- MIND website http://www.mind.org.uk/information-support/types-of-mental-health-problems/depression/#.UoTxdBZLqs9

If you are interested in learning more about ***anxiety***, you may wish to consult:

- NHS website   http://www.nhs.uk/conditions/Anxiety/Pages/Introduction.aspx
- MIND website http://www.mind.org.uk/information-support/types-of-mental-health-problems/anxiety-and-panic-attacks/#.UoTyBhZLqs8

If you would like to receive a summary of the findings when it is completed or have questions, please contact Ms Karolina Rusiak at 01248 388569 or k.rusiak@bangor.ac.uk.

If you have any complains or concerns, please address these to Mr Hefin Francis, School Manager, School of Psychology, Bangor University, Gwynedd, LL57 2AS.

Thank you very much for participating!