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Neurometabolites and associations with cognitive deficits in mild cognitive impairment: a magnetic resonance spectroscopy study at 7 Tesla

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ABSTRACT

The levels of several brain metabolites were investigated in the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) in 13 healthy controls (HC) and 13 patients with mild cognitive impairment (MCI) using single-voxel magnetic resonance spectroscopy at 7T. Levels of γ-aminobutyric acid (GABA), glutamate (Glu), glutathione (GSH), N-acetylaspartylglutamate (NAAG), N-acetylaspartate (NAA), and myo-inositol (mI) were quantified relative to total creatine (tCr). The effect of diagnosis on metabolite levels, and relationships between metabolite levels and memory and executive function, correcting for age, were investigated. MCI patients showed significantly decreased GABA/tCr (ACC, PCC), Glu/tCr (PCC), and NAA/tCr (PCC), and significantly increased mI/tCr (ACC). In the combined group, worse episodic verbal memory performance was correlated with lower Glu/tCr (PCC), lower NAA/tCr (PCC), and higher mI/tCr (ACC, PCC). Worse verbal fluency performance was correlated with lower GSH/tCr (PCC). In summary, MCI is associated with decreased GABA and Glu, most consistently in the PCC. Further studies in larger patient samples should be undertaken to determine the utility of 7T magnetic resonance spectroscopy in detecting MCI-related neurochemical changes.

1. Introduction

Alzheimer's disease (AD) is the most common neurodegenerative disease. The cognitive decline and neuropsychiatric symptoms associated with AD lead to increased disability, mortality, and caregiver burden (Alzheimer's Association, 2015; Reitz et al., 2011). The economic impact of AD is estimated to be $100B per year in the United States alone. The estimated global AD prevalence of 24 million in 2011 is projected to double every 20 years until 2040 due to the world's aging population, challenging health care systems and societies in unprecedented ways (Brookmeyer et al., 2007; Reitz et al., 2011). Mitigating the future impact of AD will depend on identifying individuals at risk, determining predictors of progression, and providing early preventive treatment in preclinical stages with high conversion rates to AD, such as mild cognitive impairment (MCI).

The complex pathophysiology of MCI/AD includes deposition of amyloid-β plaques (Aβ) in multiple brain regions including the anterior cingulate cortex (ACC) and posterior cingulate cortex (PCC) (Pike et al., 2007; Small et al., 2006), deposition of tau tangles in the medial-temporal lobe (Pike et al., 2007; Small et al., 2006), and increased oxidative stress (Butterfield, 2002; Lin and Beal, 2006; Mecocci, 2004; Wang et al., 2014) and mitochondrial dysfunction (Devi et al., 2006; Lin and Beal, 2006; Reddy and Beal, 2008) that may lead to disruptions in several neurotransmitter systems and neuronal dysfunction. The regions affected by Aβ and tau play an integral role in cognitive processes such as memory and learning, but the role of other aspects of pathophysiology in cognitive decline and the transition from MCI to AD is poorly understood.

Hypotheses with a focus on the relationships between AD pathology, mitochondrial dysfunction, and oxidative stress can be tested in vivo due to advances in molecular imaging methods. The spatial

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distribution patterns of protein deposition and associations with cognitive decline and disease progression have been studied with positron emission tomography (PET). As a complementary molecular quantification technique, proton magnetic resonance spectroscopy (MRS) can be used to determine levels of endogenous brain metabolites noninvasively in the living brain. At lower field strength (<3 T), only metabolites with high signal-to-noise ratio (SNR) can be reliably determined (without advanced separation methods such as *J*-difference editing), including the neuronal marker N-acetylaspartate (NAA) (Rae, 2014), and myo-inositol (mI), which acts as an osmolyte (Rae, 2014) and is thought to reflect demyelination, inflammation, and glial activation (Chang et al., 2013). Decreased levels of NAA and increased levels of mI are a consistent finding in MCI and AD (Gao and Barker, 2014), and have been suggested as an early biomarker for diagnosis and progression (Waragai et al., 2017).

At high field strengths (7T and above), the increased spectral resolution and SNR of MRS permits the assessment of several biochemically important molecules with enhanced sensitivity compared to 3T due to their low concentrations and coupled, often overlapped resonances (Mekle et al., 2009; Pradhan et al., 2015). These metabolites include the inhibitory neurotransmitter γ-aminobutyric acid (GABA), the redox compound glutathione (GSH), and the glutamatergic modulator N-acetylaspartylglutamate (NAAG). In the context of MCI, altered levels of GSH may indicate a challenge of the cellular redox regulation systems in response to the increased production of reactive oxygen species—for example, in the form of a depletion of the antioxidant defense line, resulting in decreased GSH levels (Mandal et al., 2015), or an adaptive upregulation, represented by increased GSH levels (Duffy et al., 2014). Abnormal levels of GABA and Glu may reflect disturbed inhibitory and excitatory neurotransmission, which primarily shape cortical information processing, and may underlie the observed loss of cognitive and executive function in MCI (Huang et al., 2017). Finally, altered levels of NAAG may exert an additional critical modulatory influence on glutamatergic neurotransmission (Jaarsma et al., 1994; Labak et al., 2010). Determining the levels of multiple low-concentration compounds in a single MRS experiment at 7T is a promising avenue to probe several critical mechanisms of MCI/AD pathophysiology (oxidative stress, disturbed neurotransmission) in vivo, and whether they are related to quantitative measures of cognitive performance.

In this study, MRS data were acquired at 7T in individuals with MCI and healthy elderly controls in two cortical regions relevant to MCI/AD pathophysiology: the ACC and PCC. The ACC and PCC were chosen as these regions have been implicated in MCI/AD in studies of neural circuitry and neuropathology that show associations between pathology in these regions and cognitive deficits and neuropsychiatric symptoms (Arnold et al., 1991; Hirao et al., 2015). Levels of the neurotransmitters GABA and glutamate (Glu), the antioxidant GSH, the neuromodulator NAAG, the neuronal marker NAA, and the osmolyte and glial marker mI were tested for differences between healthy controls (HC) and individuals with MCI. In addition, associations between metabolite levels and cognitive measures of episodic verbal memory and verbal fluency that are affected in MCI were investigated.

2. Material and methods

2.1. Subjects

2.1.1. Recruitment

Thirteen HC subjects (7 female; mean age 63.6 ± 7.8 years) and 13 patients with MCI (3 female; mean age 69.6 ± 7.7 years: $p < 0.05$ compared to HC) were included in this study.

Participants were recruited from advertisements in the community or from the Johns Hopkins University Alzheimer's Disease Research Center (2 controls and 2 MCI subjects). All subjects underwent the same diagnostic procedures. Psychiatric and cognitive evaluations included a structured clinical interview by a clinical psychologist (Structured Clinical Interview for DSM-IV) (First et al., 1995), Clinical Dementia Rating (CDR) scale (Morris, 1993), and Mini-Mental State Examination (MMSE) (Folstein et al., 1975). All participants also underwent a physical and neurological examination, laboratory testing (including complete blood count and blood chemistries), and toxicology screening (psychotropic drugs and drugs of abuse) before the MR scans. Participants were excluded from the study if they had a history of or active neurological or Axis I psychiatric disorders including substance abuse, if they were not medically stable (i.e., if they had poorly controlled hypertension and/or insulin dependent diabetes), after a positive toxicology screening for psychotropic drugs or medications with central nervous system effects, or if they used prescription or over-the-counter medications with potential central nervous system effects (e.g., antihistamines, cold medications) within the past two weeks before enrollment. The MCI patients were required to have a CDR global score of 0.5, whereas the controls were required to have a CDR global score of 0 (normal). Furthermore, all participants also underwent Aβ imaging with PET to determine whether the MCI patients had evidence of a biomarker associated with AD, in addition to meeting clinical criteria for MCI (see section 2.1.3). The study protocol and consent forms were approved by the Institutional Review Board of the Johns Hopkins University School of Medicine. Written informed consent was obtained.

2.1.2. Cognitive testing

Three measures sensitive to global cognition, executive function, and memory were selected from a comprehensive, multidomain neuropsychological assessment battery to be tested for correlations with MRS measures of metabolite levels: the MMSE; the letter fluency test included in the Delis-Kaplan Executive Function System (D-KEFS) test (Delis et al., 2001) (letter-total words recalled); and the California Verbal Learning Test (CVLT; sum of the first five recall trials) (Delis et al., 2000). The Brief Visuospatial Memory Test-Revised was also administered to assess memory deficits (Benedict, 1997).

2.1.3. Beta amyloid PET data acquisition and processing

The radiotracer (N-methyl-[^{11}C])2-(4′-methylaminophenyl)-6-hydroxybenzothiazole ([^{11}C]-PiB) was used to measure Aβ deposition in all subjects and was synthesized as previously described (Klunk et al., 2004). PET scans were acquired at the PET Center, Russell H. Morgan Department of Radiology, Johns Hopkins University School of Medicine. The scanner used was a second-generation High-Resolution Research Tomograph scanner (HRRT, Siemens Healthcare, Knoxville, TN), a cerium-doped lutetium oxyorthosilicate (Lu25i05[Ce] or LSO) based, dedicated brain PET scanner with 2 mm resolution (Sossi et al., 2005). Dynamic scanning began immediately upon a 15 mCi ±10% radiotracer injection and lasted for 90 minutes. [^{11}C]-PiB was analyzed using the simplified reference tissue model method with the cerebellum as the input function that has been validated against arterial blood sampling (Price et al., 2005; Zhou et al., 2003).

2.2. MRS data acquisition and processing

2.2.1. MRS acquisition

All data were acquired on a 7T Philips Achieva scanner (Philips Healthcare, Best, The Netherlands) with a 32-channel receive and quadrature transmit head coil (Nova Medical, Wilmington, MA). After a high-resolution (0.96 mm isotropic) anatomical T_1-weighted magnetization prepared gradient echo scan, MRS voxels were

prescribed in two brain regions: ACC and PCC (as shown in Fig. 1). For both regions, the MRS voxels had dimensions of 28 mm (anterior-posterior) × 16 mm (left-right) × 20 mm (caudal-cranial). Voxels were centered at the midline with the anterior-posterior edge tangential to the corpus callosum. The ACC voxel was placed in dorsal ACC with the caudal-cranial edge perpendicular to the genu of the corpus callosum, and the PCC voxel was placed in PCC with the caudal-cranial edge perpendicular to the splenium of the corpus callosum. Before data acquisition, shimming was performed up to 2nd order using a FASTMAP-based routine, and RF power was optimized on the localized volume. Data were acquired using the Stimulated Echo Acquisition Mode sequence with the following parameters: repetition time = 3000 ms; 96 averages; 2048 data points; 3 kHz spectral width; VAPOR (Tkac et al., 1999) water suppression. Four water-unsuppressed averages per voxel were recorded with the same settings. Echo time was set to the shortest possible value (14 ms in ACC, 15 ms in PCC).

2.2.2. Data processing

Spectroscopic data were analyzed with LCModel v6.3-0D (Provencher, 2001, 1993), using TE-specific simulated basis sets including alanine (Ala), aspartate (Asp), creatine (Cr), GABA, glucose (Glc), Glu, glutamine (Gln), GSH, glycerophosphocholine (GPC), glycine (Gly), lactate (Lac), mI, NAA, NAAG, phosphocholine (PCh), phosphocreatine (PCr), phosphoethanolamine (PE), serine (Ser), scyllo-inositol (sI), taurine (Tau), and resonances from lipids (Lip09, Lip13a-d, Lip20) and macromolecules (MM09, MM12, MM14, MM17, MM20) as internally simulated by LCModel. The levels of GABA, Glu, GSH, NAA, NAAG, and mI (estimated with respect to the total creatine signal tCr = Cr + PCr) were used for further analysis. Individual metabolite measures with Cramér-Rao lower bounds (as determined by LCModel) higher than 15% were excluded. Detection of gross outliers was performed by calculating the mean Cook's distance across both groups (HC, MCI) and regions (ACC, PCC) for each metabolite (Stevens, 1984). Individual data points with more than 5 times the mean Cook's distance were regarded as gross outliers and discarded.

The T_1-weighted structural images were segmented with SPM12 (Friston, 2007) into separate gray matter (GM), white matter (WM) and cerebrospinal fluid (CSF) probability maps. Using an in-house-developed MATLAB (The MathWorks, Natick, MA) routine based on the Gannet 3.0 toolbox (Edden et al., 2014), the MRS voxels for each participant were coregistered to the structural image, and the relative fractions of voxel tissue composition for GM, WM, and CSF were extracted. GM tissue fraction was subsequently used as a covariate (see below) to account for the individual degree of GM loss, which may underlie changes in metabolite levels.

2.2.3. Statistical analysis

All statistical analyses were performed in R (R core team, 2017). For each metabolite, a two-way analysis of covariance was performed on the entire data set after exclusion and outlier rejection. Metabolite levels (expressed as ratios to tCr) were defined as dependent variables and group (HC, MCI) and region (ACC, PCC) as independent variables. To investigate effects of group, post-hoc pairwise contrasts between HC and MCI groups were tested using

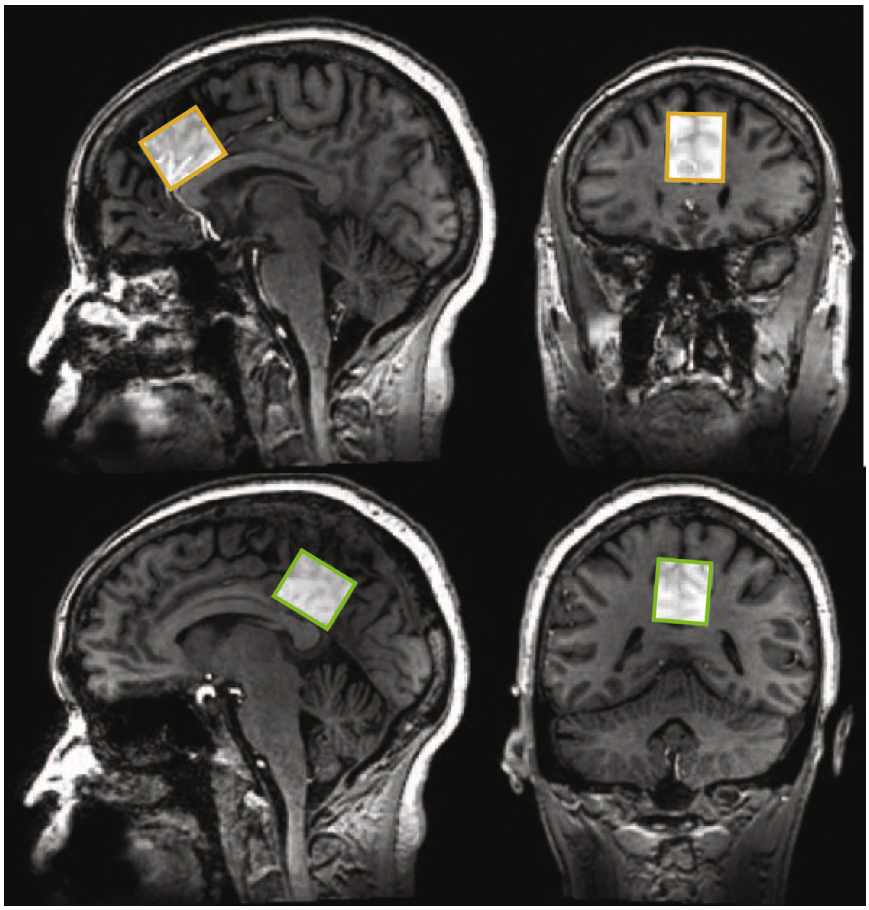

Fig. 1. Voxel placement in the ACC (upper panel) and PCC (lower panel). For both voxels, dimensions were 28 mm (anterior-posterior) × 16 mm (left-right) × 20 mm (caudal-cranial). Abbreviations: ACC, anterior cingulate cortex; PCC, posterior cingulate cortex.

the least squares means method for each region. Tukey-adjusted comparisons were tested for significance at a single-test alpha level of 0.05. To assess relationships between local metabolite levels and cognitive scores, separate linear regression analyses were performed between each metabolite and cognitive score in ACC and PCC, respectively, at a single-test alpha level of 0.05.

For the two-way analysis of covariance modeling and the linear regression analyses, models were designed with the following covariates: (1) without covariates; (2) age; (3) age and GM tissue fraction; (4) age and sex; and (5) age, sex, and GM tissue fraction. The linear regression analyses showed the best adjusted R^2 (adjusted for the number of covariates) for the model only including age as a covariate (see results for all models in the Supplementary Material), which was subsequently chosen for analysis.

To investigate whether changes in metabolites correlated with the extent of neuronal loss characteristic of MCI, relationships between metabolite levels and levels of NAA (which is generally interpreted as a general indicator of neuronal integrity) were assessed separately for each voxel using Pearson's product-moment correlation analysis. The rationale for this additional analysis was to assess whether potential changes in levels of neurotransmitters or oxidative compounds can be linked to the specific mechanism of neuronal loss, or whether they appear independent, for example, as a result of altered metabolic synthesis or recycling mechanisms.

To scrutinize whether disease effects of the reference compound (tCr) are driving changes in metabolite-to-tCr ratios, the signal ratios of tCr to internal tissue water in institutional units (as returned by LCModel with the water relaxation correction parameter "atth2o" = 0.742) were compared between groups (separately for ACC and PCC) with a two-tailed *t*-test at a single-test alpha level of 0.05.

3. Results

All MCI participants demonstrated a distribution volume ratio for [^{11}C]-PiB that is associated with cognitive decline; 1.2 or higher for anterior (anterior cingulate or middle frontal cortex) and/or posterior cortical regions (Resnick et al., 2010) (superior temporal cortex, precuneus or posterior cingulate; data not shown). In addition, all MCI patients performed at least one and a half standard deviations below the normative value on either the CVLT or the Brief Visuospatial Memory Test (data not shown). Thus, based on the Aβ imaging and cognitive performance, this MCI sample is likely to demonstrate further cognitive decline.

Of 52 data sets (26 subjects × 2 voxels), the following numbers were discarded before statistical analysis: 6 for GABA (1 outlier); 2 for Glu (2 outliers); 5 for GSH (2 outliers); 2 for NAA (2 outliers); 21 for NAAG (2 outliers); 1 for mI (1 outlier). Representative spectra and LCModel fits for ACC and PCC are shown in Fig. 2, indicating a good SNR, low linewidth, and successful water suppression.

Mean MMSE were 28.7 ± 1.2 for HC and 27.5 ± 1.7 for MCI ($p < 0.01$). Mean D-KEFS scores were 42.8 ± 12.6 for HC and 36.4 ± 12.5 for MCI ($p = 0.073$). Mean CVLT (sum of the first five trials) were 55.5 ± 8.3 for HC and 38.5 ± 10.3 for MCI ($p < 0.001$).

Mean GM tissue fractions were 64 ± 7 % for HC and 59 ± 7 % for MCI in ACC ($p = 0.12$), and 64 ± 12 % for HC and 60 ± 6 % for MCI in PCC ($p = 0.38$). Mean WM tissue fractions were 11 ± 5 % for HC and 15 ± 9 % for MCI in ACC ($p = 0.23$), and 21 ± 6 % for HC and 24 ± 3 % for MCI in PCC ($p = 0.09$). Mean CSF tissue fractions were 25 ± 9 % for HC and 26 ± 10 % for MCI in ACC ($p = 0.83$), and 16 ± 10 % for HC and 16 ± 4 % for MCI in PCC ($p = 0.96$).

Mean tCr/water was 3.97 ± 0.35 i.u. for HC and 3.95 ± 0.38 i.u. for MCI in ACC ($p = 0.89$), and 4.44 ± 0.40 i.u. for HC and 4.63 ± 0.39 % for MCI in PCC ($p = 0.25$).

Group comparisons between the HC and MCI groups revealed statistically significant differences in metabolite-to-tCr ratios (Table 1). MCI patients had significantly lower levels of GABA/tCr (ACC and PCC, $p < 0.01$ each), Glu/tCr (PCC, $p < 0.05$), and NAA/tCr (PCC, $p < 0.05$), and significantly higher levels of mI/tCr (ACC, $p < 0.01$). If no covariates were included in the models, Glu/tCr in the ACC was significantly lower in MCI patients ($p < 0.05$). No additional significant results were observed when including sex or GM content as covariates (see Supplementary Material).

MMSE test scores showed a negative correlation with mI/tCr in the ACC ($p < 0.01$) and the PCC ($p < 0.05$). D-KEFS scores were

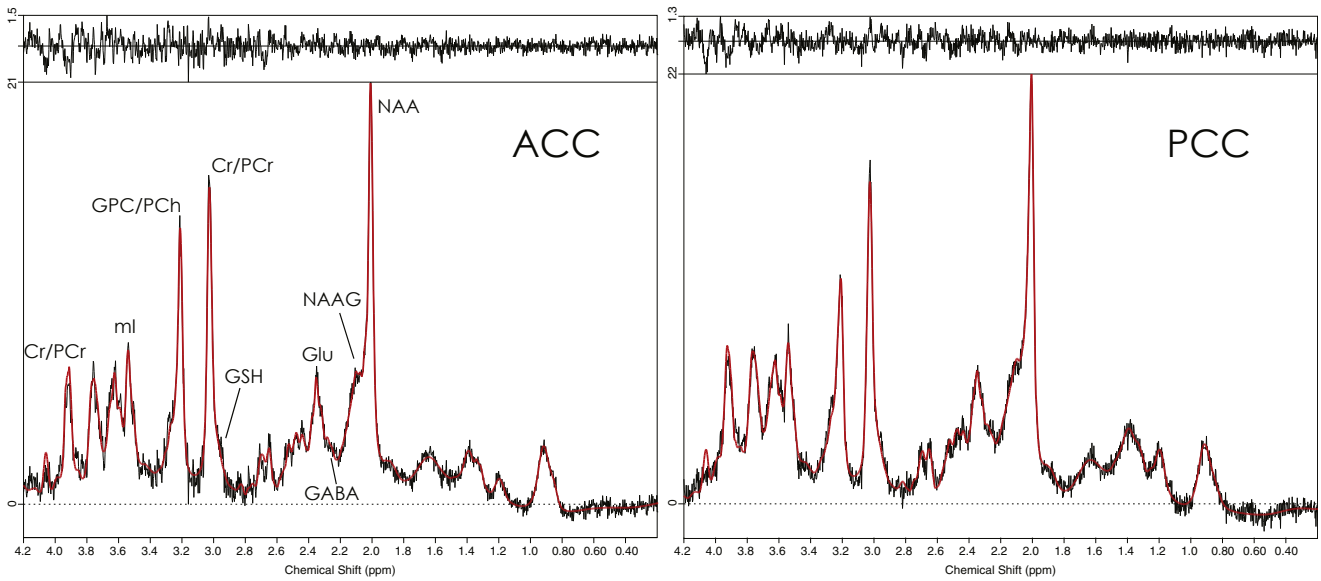

Fig. 2. Representative spectra (black) and LCModel fits (red) for STEAM data from ACC (left panel) and PCC (right panel). Fit residuals are shown at the top of the respective panel. Abbreviations: ACC, anterior cingulate cortex; PCC, posterior cingulate cortex; GABA, γ-aminobutyric acid; Glu, glutamate; GSH, glutathione; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; mI, myo-inositol; Cr, creatine; GPC, glycerophosphocholine; PCh, phosphocholine; PCr, phosphocreatine. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Quantitative results of post hoc group comparisons after two-way ANCOVA analysis, with metabolite levels as dependent variable and group and region as independent variables, and age as covariate

Metabolite [/tCr]	ACC			PCC		
	HC	MCI	*p*	HC	MCI	*p*
GABA	0.418 (0.018)	0.343 (0.017)	**0.005**	0.361 (0.017)	0.295 (0.016)	**0.009**
Glu	1.418 (0.025)	1.340 (0.023)	0.096	1.292 (0.023)	1.215 (0.023)	**0.024**
GSH	0.215 (0.011)	0.208 (0.010)	0.685	0.233 (0.011)	0.230 (0.010)	0.835
NAA	1.440 (0.028)	1.374 (0.027)	0.095	1.542 (0.028)	1.435 (0.027)	**0.010**
NAAG	0.209 (0.015)	0.197 (0.013)	0.551	0.230 (0.012)	0.206 (0.010)	0.163
mI	0.662 (0.018)	0.750 (0.018)	**0.001**	0.685 (0.018)	0.732 (0.018)	0.072

Key: ACC, anterior cingulate cortex; GABA, γ-aminobutyric acid; Glu, glutamate; GSH, glutathione; HC, healthy control; MCI, mild cognitive impairment; mI, myo-inositol; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; PCC, posterior cingulate cortex.
Results were tested for statistical significance at a single-test alpha level of 0.05. The table shows the group mean with the standard error of the mean (SEM) in parentheses. Bold values indicates $P < 0.05$.

positively correlated with GSH/tCr in the PCC ($p < 0.01$). CVLT scores were positively correlated with Glu/tCr (PCC, $p < 0.05$) and NAA/tCr (PCC, $p < 0.05$) and negatively correlated with mI/tCr (ACC and PCC, $p < 0.05$ each). Results of the analyses of relationships between metabolite levels and cognitive scores are summarized in Table 2. Without covariates included in the models, positive correlations between CVLT scores and Glu/tCr and NAA/tCr were observed in the ACC ($p < 0.05$ each), and mI/tCr did not correlate with any test score. None of the additional correlations were observed after inclusion of GM and sex as covariates. (see Supplementary Material). Glu/tCr was positively correlated with NAA/tCr in ACC and PCC ($p = 0.001$ each). No other relationships of metabolite levels with NAA/tCr were observed (Table 3).

4. Discussion

This study investigated differences in the levels of several brain metabolites between healthy elderly subjects and MCI patients, as well as their relationships with measures of cognitive functioning. To the best of our knowledge, this is the first study to use MRS at 7T field strength to study metabolic changes in MCI patients, including quantification of the low-concentration metabolites GABA, GSH, and NAAG in a single session. In addition to reproducing well-known findings from lower field strengths (i.e., decreased NAA and increased mI), this study also found selective reductions in the neurotransmitters GABA and Glu. The MCI patients studied are likely to demonstrate subsequent cognitive decline based on memory deficits and cortical beta amyloid levels. Furthermore, the MCI patients were carefully screened for psychiatric and medical comorbidities that could be associated with cognitive impairment in addition to AD.

One of the interesting significant findings was a reduction of ~16% in both ACC and PCC GABA/tCr levels in MCI patients compared to controls. No relationships between GABA/tCr levels and cognitive scores were found, however. These observations are in line with several previous findings from studies of MCI and AD patients that used *J*-difference–edited MRS at 3T. Most prominently, decreased posterior cingulate GABA+ macromolecules (GABA+) levels were found in patients with amnestic MCI (Riese et al., 2015). In that study, no relationship between GABA+ and MMSE (but with the CERAD word learning score) or the uptake of the Aβ radiotracer Pittsburgh-B (PiB) PET compound was observed, indicating that GABA+ decrease is not directly related to the extent of Aβ deposition. Similarly, another study found significantly lower levels of GABA+ in AD patients for the posterior, but not the anterior cingulate region (Bai et al., 2014). As in the present study, levels

Table 2
Results of linear regression analysis for relationships between metabolite levels and cognitive test measures for the combined group (both control and MCI subjects)

Metabolite [/tCr]	ACC			PCC		
	MMSE	D-KEFS	CVLT	MMSE	D-KEFS	CVLT
GABA	$R^2 = 0.403$	$R^2 = -0.069$	$R^2 = 0.109$	$R^2 = 0.180$	$R^2 = -0.035$	$R^2 = 0.093$
	$p = 0.685$	$p = 0.806$	$p = 0.114$	$p = 0.709$	$p = 0.829$	$p = 0.207$
	$p_{age} = 0.001$	$p_{age} = 0.458$	$p_{age} = 0.315$	$p_{age} = 0.014$	$p_{age} = 0.301$	$p_{age} = 0.117$
Glu	$R^2 = 0.064$	$R^2 = 0.006$	$R^2 = 0.155$	$R^2 = 0.063$	$R^2 = 0.105$	$R^2 = 0.207$
	$p = 0.884$	$p = 0.839$	$p = 0.065$	$p = 0.114$	$p = 0.325$	$\mathbf{p = 0.022}$
	$p_{age} = 0.114$	$p_{age} = 0.234$	$p_{age} = 0.558$	$p_{age} = 0.458$	$p_{age} = 0.160$	$p_{age} = 0.611$
GSH	$R^2 = 0.209$	$R^2 = 0.013$	$R^2 = 0.030$	$R^2 = 0.253$	$R^2 = 0.361$	$R^2 = 0.074$
	$p = 0.978$	$p = 0.259$	$p = 0.637$	$p = 0.092$	$\mathbf{p = 0.002}$	$p = 0.356$
	$p_{age} = 0.011$	$p_{age} = 0.326$	$p_{age} = 0.137$	$p_{age} = 0.010$	$p_{age} = 0.065$	$p_{age} = 0.079$
NAA	$R^2 = 0.085$	$R^2 = -0.037$	$R^2 = 0.139$	$R^2 = 0.050$	$R^2 = -0.037$	$R^2 = 0.180$
	$p = 0.509$	$p = 0.821$	$p = 0.069$	$p = 0.598$	$p = 0.747$	$\mathbf{p = 0.041}$
	$p_{age} = 0.087$	$p_{age} = 0.337$	$p_{age} = 0.276$	$p_{age} = 0.146$	$p_{age} = 0.296$	$p_{age} = 0.364$
NAAG	$R^2 = 0.209$	$R^2 = 0.021$	$R^2 = -0.131$	$R^2 = 0.234$	$R^2 = 0.023$	$R^2 = -0.065$
	$p = 0.753$	$p = 0.170$	$p = 0.661$	$p = 0.264$	$p = 0.378$	$p = 0.667$
	$p_{age} = 0.067$	$p_{age} = 0.445$	$p_{age} = 0.417$	$p_{age} = 0.019$	$p_{age} = 0.187$	$p_{age} = 0.383$
mI	$R^2 = 0.346$	$R^2 = 0.102$	$R^2 = 0.191$	$R^2 = 0.241$	$R^2 = -0.028$	$R^2 = 0.155$
	$\mathbf{p = 0.005}$	$p = 0.117$	$\mathbf{p = 0.034}$	$\mathbf{p = 0.030}$	$p = 0.566$	$\mathbf{p = 0.050}$
	$p_{age} = 0.005$	$p_{age} = 0.072$	$p_{age} = 0.035$	$p_{age} = 0.014$	$p_{age} = 0.279$	$p_{age} = 0.059$

Key: ACC, anterior cingulate cortex; CVLT, California Verbal Learning Test; D-KEFS, Delis-Kaplan Executive Function System; GABA, γ-aminobutyric acid; Glu, glutamate; GSH, glutathione; MCI, mild cognitive impairment; mI, myo-inositol; MMSE, Mini Mental State Examination; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; PCC, posterior cingulate cortex.
p values represent partial correlations between metabolite levels (or age) and cognitive test measures. R^2 is the adjusted value for the complete linear regression model including age as covariate. Partial correlations between metabolite levels and cognitive test measures were considered significant at a single-test alpha level of 0.05.
Bold values indicates $P < 0.05$.

Table 3
Results of linear regression analysis for relationships between metabolite levels and NAA levels as marker of neuronal integrity for the combined group (both control and MCI subjects)

Metabolite [/tCr]	Correlation with NAA/tCr ACC	Correlation with NAA/tCr PCC
GABA	R = 0.098	R = 0.167
	$p = 0.672$	$p = 0.425$
Glu	R = 0.615	R = 0.595
	$p = 0.001$	**$p = 0.001$**
GSH	R = 0.302	R = −0.199
	$p = 0.161$	$p = 0.351$
NAAG	R = 0.050	R = 0.254
	$p = 0.878$	$p = 0.295$
mI	R = 0.011	R = −0.014
	$p = 0.957$	$p = 0.947$

Key: ACC, anterior cingulate cortex; GABA, γ-aminobutyric acid; Glu, glutamate; GSH, glutathione; mI, myo-inositol; NAA, N-acetylaspartate; NAAG, N-acetylaspartylglutamate; PCC, posterior cingulate cortex; tCr; total creatine.
R represents the Pearson product moment correlation coefficient. Correlations between metabolite levels were considered significant at a single-test alpha level of 0.05. Note that the lack of general correlation indicates that effects are not primarily driven by changes in the tCr reference signal.
Bold values indicates $P < 0.05$.

of GABA were not correlated with MMSE. Another study did not find significant group differences for GABA+ or Glu+Gln (Glx) between healthy subjects, MCI, and AD patients in the ACC and right hippocampus, nor were correlations with MMSE or the Montreal Cognitive Assessment score revealed (Huang et al., 2017). In a study of healthy elderly subjects and MCI patients using J-PRESS at 3T, it was found that administration of human growth hormone factor led to significant increases of GABA levels in dorsolateral frontal, posterior cingulate, and posterior parietal regions, which coincided with, but was not correlated with, improved cognitive functioning (Friedman et al., 2013). The finding in the present study of decreased GABA in ACC suggests that disturbed GABA homeostasis in MCI may also occur in cortical regions other than the PCC, with potential impact on cognitive function. The discrepancy to previous studies may be explained by the increased specificity of GABA detection in unedited MRS at 7T, compared to *J*-difference–edited methods at 3T that usually report combined GABA and macromolecules in a larger measurement volume.

Glu (or Glx) levels in MCI/AD have been more frequently studied, as the relatively strong combined Glx signal is easily detectable even with standard MRS techniques at lower field strengths. As the present study benefits from the higher field strength of 7T, the discrimination between Glu and Gln is improved, and the Glu estimates can be assumed to be more reliable and specific than at 1.5–3T, revealing reduced Glu/tCr levels in MCI (by ~5–6%). The observed correlation between PCC Glu/tCr and CVLT scores may suggest that disturbed Glu neurotransmission is at least partly contributing to memory impairment in MCI. A recent study found reduced posterior cingulate Glu levels in patients with amnestic MCI, which also inversely correlated with global PiB uptake (Zeydan et al., 2017). Previous studies reported decreased Glx levels in the posterior cingulate in MCI compared to controls (Hattori et al., 2002); no decrease in MCI, but in patients with AD in the posterior cingulate (Fayed et al., 2011) and hippocampus (Rupsingh et al., 2011); no differences between MCI and controls, but longitudinal changes over time in atypical MCI patients in the posterior cingulate (Olson et al., 2008).

Reduced GABA or Glu levels may indicate loss of GABA or glutamatergic neurons per se, disturbances in GABA/Glu synthesis and/or changes in the Glu/Gln/GABA cycle between astrocytes and neurons. Although changes in Glu/tCr levels in this study were closely linked to changes in levels of the neuronal marker NAA/tCr, GABA/tCr levels did not show a relationship with NAA/tCr. This finding may suggest that Glu changes are largely caused by the specific loss of glutamatergic neurons. In contrast, the notable decrease in bulk GABA/tCr levels may rather be attributed to MCI-induced alterations in GABA synthesis or recycling.

Due to the inherent difficulties in reliably differentiating NAAG from NAA, NAAG has not been extensively studied to date. Even at 7T, the separation of the two compounds relies on optimal measurement conditions, as evidenced by the relatively large fraction of data sets that were excluded due to high Cramér-Rao lower bounds values for NAAG. The present study is the first study to explicitly compare NAAG levels between healthy subjects and MCI patients at 7T, observing no effects of disease or correlations with cognitive measures (albeit in a substantially reduced cohort for aforementioned data quality reasons). A postmortem study revealed decreased NAAG levels in AD brains (Jaarsma et al., 1994), and increased NAAG levels were reported in the dorsolateral prefrontal cortex after human growth hormone factor treatment (Friedman et al., 2013). However, the functional significance of NAAG remains not well understood, beyond a putative role as neuromodulator activating secondary messenger pathways via glutamatergic metabotropic activity (Neale, 2011), and as a potential source of Glu. Furthermore, the decrease in NAAG may be observed in AD rather than in its preclinical stages such as MCI.

In contrast, decreased levels of NAA in several brain areas in MCI/AD have been long established (Gao and Barker, 2014), which is primarily thought of as an (unspecific) indicator of neuronal loss. However, reduced NAA seems to be more closely related to clinical disease progression than is cerebral atrophy (Adalsteinsson et al., 2000). Similarly, increased mI levels are a classic hallmark of MCI/AD pathophysiology, likely indicating glial activation, gliosis, and inflammation, and providing a link to protein-related AD neuropathology. Although the data in this study confirm the previously observed increase of mI/tCr in MCI patients in the ACC, the group comparison did not reach significance ($p = 0.07$) for the PCC. It is possible that investigations with a larger sample size might yield significant differences for the PCC as well, in particular, because mI/tCr was associated with cognitive scores in both regions of interest. The combined NAA/mI ratio has been suggested as an early biomarker of individuals at risk for developing MCI and progressing to AD (Waragai et al., 2017), and both compounds have been closely linked to increased accumulation of Aβ (Kantarci et al., 2011; Nedelska et al., 2017) and tau (Murray et al., 2014). In this light, the effects of NAA and mI that were observed in this study are in line with these findings; although, it is notable that mI was the only metabolite to be associated with the MMSE score, suggesting that it may serve as a functionally unspecific indicator of general disease severity. Interestingly, no direct correlation between NAA/tCr and mI/tCr levels was observed, indicating that the respective pathophysiological processes are not immediately linked.

Finally, although this study showed no group differences in GSH/tCr levels between MCI patients and controls, it revealed a relationship between PCC GSH/tCr levels and verbal fluency. Previous in vivo MRS studies of GSH are rare: one study found significantly decreased GSH levels in MCI and AD in the hippocampus, but only in AD in prefrontal areas (Mandal et al., 2015). In contrast to the present study, close relationships were found between prefrontal GSH and MMSE as well as CDR scores. Recently, associations between decreased temporal and parietal GSH levels and local PiB uptake measures were found, indicating relationships between oxidative stress and amyloidosis (Chiang et al., 2017). Taken together, these findings indicate a promising link between several pathophysiological MCI/AD mechanisms and cognitive outcome, and certainly warrant further investigation in terms of regional and functional specificity.

4.1. Limitations

This study features comparably small sample sizes with 13 individuals in each cohort. This limitation in power may have prevented the detection of significant effects of disease (e.g., ACC Glu). It should also be noted that there was a significant difference in age between the patient and control groups (with the MCI group being older). Age was therefore included as a covariate in the group analysis of metabolite levels and in the analysis of correlation between metabolite levels and cognitive scores. Finally, the strict requirements concerning spectral quality resulted in frequent rejection of quantitative NAAG measures, suggesting that the respective negative findings should be regarded with caution due to the low statistical power. Future studies investigating MCI-related changes of NAAG may require dedicated spectral editing efforts or optimized subecho times for unambiguous detection (Choi et al., 2010; Edden et al., 2007).

5. Conclusions

Results of this 7T MRS study revealed several region-specific effects of MCI on brain metabolite levels. Specifically, MCI was associated with decreased GABA and Glu, most consistently in the PCC, the region implicated by studies of cerebral glucose metabolism and Aβ deposition in MCI. Interactions between metabolite levels and cognitive scores were observed for specific brain regions. This suggests that key mechanisms in MCI/AD pathophysiology, such as oxidative stress and disturbed neurotransmission, may contribute to cognitive deficits in a highly region- and function-specific manner. Future studies of MCI and AD patients should use 7T MRS in various implicated brain regions including medial-temporal lobe/hippocampus to improve the understanding of the regional specificity of brain metabolite changes and their effects on different domains of cognitive function.

Disclosure

All authors declare that they have no actual or potential conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version at https://doi.org/10.1016/j.neurobiolaging.2018.09.027.

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