Low episodic memory performance in cognitively normal elderly subjects is associated with increased posterior cingulate gray matter N-acetylaspartate: A ¹H MRSI Study at 7 Tesla.

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1 **ABSTRACT**

2 Low episodic memory performance characterizes elderly subjects at increased risk for
3 Alzheimer's disease (AD) and may reflect neuronal dysfunction within the posterior cingulate
4 cortex and precuneus region (PCP). To investigate a potential association between cerebral
5 neuro-metabolism and low episodic memory in the absence of cognitive impairment, tissue
6 specific magnetic resonance spectroscopic imaging (MRSI) at ultra-high field strength of 7 Tesla
7 was used to investigate the PCP-region in a healthy elderly study population (n=30, age 70 ± 5.7
8 years, Mini Mental State Examination 29.4 ± 4.1). The Verbal Learning and Memory Test (VLMT)
9 was administered as part of a neuropsychological battery for assessment of episodic memory
10 performance. Significant differences between PCP-gray and white matter could be observed for
11 glutamate-glutamine ($p=0.001$), choline ($p=0.01$) and myo-inositol ($p=0.02$). Low VLMT
12 performance was associated with high N-acetylaspartate in PCP-gray matter ($p=0.01$), but not in
13 PCP-white matter. Our data suggest that subtle decreases in episodic memory performance in
14 the elderly may be associated with increased levels of NAA as a reflection of increased
15 mitochondrial energy capacity in PCP-gray matter.

1. INTRODUCTION

Low episodic memory performance in otherwise cognitively normal elderly subjects has been linked to increased risk for sporadic Alzheimer's disease (AD) (Elias, et al., 2000, Grober, et al., 2000, Linn, et al., 1995, Pasquier, 1999, B.J. Small, et al., 2000) and may reflect preclinical brain change (Albert, et al., 2011, Caselli, et al., 2007). Several studies have provided evidence on the close relationship between episodic memory dysfunction and AD pathology, as initially observable in limbic brain regions and widespread cerebral neurodegeneration at later stages (Bennett, et al., 2006, Kohler, et al., 1998, Mormino, et al., 2009, Pike, et al., 2007, Storandt, et al., 2009, Vyhnalek, et al., 2014). While AD pathology ultimately affects the entire brain, changes in the posterior cingulate and precuneus region (PCP) may have particular relevance for processing of episodic memory tasks (Brugnolo, et al., 2014, Chetelat, et al., 2003, Sperling, et al., 2010), as the PCP-region is a central hub in cognitive brain networks that include the default mode network (DMN) (Buckner, et al., 2008, Cavanna and Trimble, 2006, Raichle, et al., 2001) and also limbic nuclei, such as the hippocampus (Greicius, et al., 2004, Insel and Takehara-Nishiuchi, 2013, Wagner, et al., 2005). Moreover, functional, metabolic and structural abnormalities in the PCP characterize individuals with and at risk for AD, as reflected by mild cognitive impairment (MCI) (Chetelat, et al., 2005, Mintun, et al., 2006, Sheline, et al., 2010, G.W. Small, et al., 2000, Teipel, et al., 2015).

Metabolic brain change can be assessed non-invasively by ^1H -magnetic resonance spectroscopy (MRS), and significant alterations of brain metabolites have been reported for AD and also MCI (Kantarci, et al., 2000, Metastasio, et al., 2006, Moats, et al., 1994, Pilatus, et al., 2009, Riese, et al., 2015, Yang, et al., 2012). The metabolic markers N-acetylaspartate (NAA), total choline (tCho), myo inositol (mI), glutamate-glutamine (glx), and gamma-amino butyric acid (GABA) have been linked to various neurophysiological processes, including energy metabolism and mitochondrial capacity, lipid metabolism, inflammation, glial activation and neurotransmission (Bitsch, et al., 1999, Brand, et al., 1993, Chen, et al., 2009, Filibian, et al., 2012, Kapogiannis, et al., 2013, Moffett, et al., 2007). While reduced NAA may reflect neuronal loss in advanced AD dementia (Kantarci, et al., 2000, Klunk, et al., 1992, Moats, et al., 1994, Schuff, et al., 2002), NAA has furthermore been

suggested to possibly represent a marker for treatment response and mitochondrial capacity in a context of neuronal recovery (Ellis, et al., 1997, Jessen, et al., 2006, Olson, et al., 2008). When performing magnetic resonance spectroscopic imaging (MRSI), effectively multiple MRS voxels are acquired in an integrated way for generation of metabolic maps, which allow the investigation of specific anatomical regions or cerebral gray and white matter (MacKay, et al., 1996, Maudsley, et al., 2009, Schuff, et al., 2002, Schuff, et al., 2001). Signal-to-noise ratio (SNR) and spectral resolution significantly improve by performing MRS at high magnetic field strength (Barker, et al., 2001, Boer, et al., 2011, Mekle, et al., 2009, Pradhan, et al., 2015, Tkac, et al., 2009, Ugurbil, et al., 1993). Moreover, MRSI at ultra-high field strength may provide metabolic maps at high spatial resolution, as demonstrated for free induction decay (FID) ^1H -MRSI at 7 Tesla (7T) (Bogner, et al., 2012, Henning, et al., 2009). At this point several MRS studies have been performed on AD and MCI. However, to our knowledge, variation of memory performance in cognitively normal elderly subjects at increased risk for sporadic AD due to their age, has not been studied by MRS or MRSI. The current study therefore aims to investigate whether subtle deficits in episodic memory performance in the cognitively normal elderly may be linked to alterations of NAA and other metabolites in PCP gray and white matter by administering FID ^1H -MRSI at ultra-high field strength of 7T.

2. METHODS

2.1 Study sample

Thirty participants were recruited at the Hospital for Psychogeriatric Medicine (Gerontopsychiatrisches Zentrum Hegibach), University of Zürich, Switzerland (**Table 1**). The study was conducted in accordance with good clinical practice guidelines issued by the local ethics committee (Kantonale Ethikkommission Zürich, <http://www.kek.zh.ch>), as well as with the declaration of Helsinki. All procedures were approved by the Kantonale Ethikkommission Zürich. Written informed consent was obtained from all participants. All participants received standardized cognitive testing and a clinical work-up including medical history, blood sampling, medical and neurological examination. Inclusion criteria were age >65 years and unimpaired overall cognitive status as indicated by Mini Mental State Examination (MMSE) $\geq 27/30$, neuropsychological testing, and comprehensive psychiatric examination. Subjects were excluded in case of presence of any conditions possibly affecting cognition, including current psychotropic medication. Furthermore, subjects with any neurological or mental condition including drug abuse, claustrophobia, major vascular brain disease such as stroke or past intracranial hemorrhage as well as contraindications to MRI in general, were not included.

2.2 Cognitive Testing

The MMSE was used as a global measure of cognition (Folstein, et al., 1975). Memory performance of participants was assessed with the delayed recall measure of the Verbal Learning and Memory Test (VLMT) (Helmstaedter, 2001, Helmstaedter and Durwen, 1990), which is an adapted German language version of the Rey Auditory Verbal Learning Test (Lezak, 1983, Müller, et al., 1997). The VLMT includes serial learning and recall of a 15-item word list over five trials. Recall performance was assessed immediately after reading the word list to the participant, and also after distraction (VLMT delayed recall measure, used for further analysis in this study). Further neuropsychological testing included a short version of the Boston Naming Test (BNT) for assessment of language (Nicholas, et al., 1988, Thalmann, et al., 1997), Digit Span Test attention and short term memory (Härting, et al., 2000, Wechsler, 1987) and the ratio

between part A and part B of the Trail Making Test as a measure of executive function (Reitan, 1958, Tombaugh, 2004). Presence of significant depression was screened for by applying the Geriatric Depression Scale (GDS) (Yesavage, et al., 1982).

2.3 MRI and MRSI acquisition

All MRI scans were obtained within one session using a Philips Achieva 7 Tesla scanner (Philips Healthcare, Best, The Netherlands) with a quadrature transmit head coil and a 32-channel receive coil (Nova Medical, Wilmington, MA, USA) at the Institute for Biomedical Engineering, ETH and University of Zurich, Switzerland. For localization of the MRSI volume of interest (VOI), tissue segmentation and definition of region of interest (ROI), a high resolution T1-weighted 3D magnetization prepared rapid gradient echo (MPRAGE) structural image (TE = 3.74 ms; TR = 8.22 ms; scan mode = 3D; resolution [x, y, z] = 0.8 x 0.8 x 0.9 mm; scan time = 10:54 min) and a 3D-fluid-attenuated inversion recovery (FLAIR) sequence (TE = 310.74 ms; TR = 8000 ms; scan mode = 3D; resolution [x, y, z] = 0.43 x 0.43 x 2 mm; scan time = 7:00 min) were collected prior to MRSI. A high-resolution direct free induction decay (FID) MRSI sequence (acquisition delay = 2.5 ms; TR = 644 ms; scan time = 29:22 min) (Henning, et al., 2009) was acquired without outer volume or fat suppression (Bogner, et al., 2012), and combined with a water suppression scheme similar to VAPOR (Tkac, et al., 1999). The in-plane resolution of the MRSI of 3.5×3.5 mm², determined by a 57×48 grid over a 200×168 mm² FOV, and slice thickness of 12 mm yielded a nominal voxel size of 0.15 ml. The MRSI slab was located above the lateral ventricles and corpus callosum, and slightly angulated towards the occipital lobe using the MPRAGE image for anatomical guidance. For exact anatomical reference of the MRSI, a T1-weighted structural turbo spin-echo image (TE = 2.9 ms; TR = 5.7 ms; resolution [x, y, z] = 0.9 x 0.9 x 1.5 mm; scan time = 28.2 seconds) was obtained using the same field of view (FOV) dimensions as for the FID MRSI. Prior to MRSI acquisition, a B₀ map was obtained, and localized third-order B₀ shimming was applied to reduce magnetic field inhomogeneities, especially accounting for the absence of outer volume and fat suppression. To that end, the active B₀ shimming procedure utilized an anatomically shaped shim-ROI and in addition a region of less interest (ROLI), both manually

defined by the operator (M.W. and S.J.S.), combined with the application of multiple sets of starting values (MSV). This combined constrained ROLI-MSV approach for MRSI has been shown to significantly improve spectral quality in high-resolution FID MRSI at ultra-high field strength (Fillmer, et al., 2015). All MR scans were visually inspected by a trained neurologist (P.G.U.) for atrophy of brain tissue, presence of cerebral infarction, tumors, or microhemorrhage

2.4 MRI data processing and ROI definition

Volumetric analysis of relevant brain structures was performed with FreeSurfer Version 5.1 (<http://surfer.nmr.mgh.harvard.edu>) (Fischl, et al., 2002, Fischl, et al., 2004, Fischl, et al., 1999), as performed earlier by our group (Schreiner, et al., 2014). Briefly, registration to a probabilistic brain atlas, derived from manually labeled brain scans, was used for algorithm-based parcellation to assign neuroanatomical labels to each voxel in the MRI volume. Left and right hippocampal volumes were used as sensitive indicators of brain change related to AD (Frisoni, et al., 2010). Total gray matter volume and sizes of the left and right lateral ventricles were used as indicators of general brain atrophy.

To estimate tissue and PCP-specific measures of MRSI, custom ROIs of the PCP gray and white matter, corresponding to the FOV and spatial resolution of the MRSI, were generated for each subject. Custom ROIs were based on tissue probability maps, a co-registered anatomical reference atlas (<http://www.brain-development.org>), and the structural MRI of the MRSI slab (sMRI). All computational procedures were conducted within the environment of SPM12 (<http://www.fil.ion.ucl.ac.uk/spm>), and Matlab (Version 8.5.0, MathWorks Inc., Natick, MA, USA). Figure 1 shows the sequential post-processing steps. FLAIR and sMRI images were co-registered to the corresponding MPAGE volume using default settings in the SPM co-register function. Next, the SPM segmentation algorithm (default settings) was used to generate tissue probability maps indicating gray matter and white matter probability (Ashburner and Friston, 2005). Optimal separation of tissue classes was achieved using multi-channel segmentation of MPAGE and co-registered FLAIR images, yielding increased image contrast at the outer borders of the brain (van Veluw, et al., 2015). The SPM segmentation procedure with standard Montreal

Neurological Institute (MNI) templates provided a transformation matrix, M^{norm} , defining the inverse normalization parameters from MNI to each subject's native space.

To assess MRSI metabolites in the PCP, the Hammers atlas, which is a three-dimensional maximum probability atlas of the human brain containing 83 anatomical labels (Gousias, et al., 2008, Hammers, et al., 2003), was modified in-house to generate a binary mask of the PCP. This ROI comprised the posterior cingulate and precuneus, corresponding to the Brodmann areas BA 31 and medial parts of BA 7. The superior and inferior anatomical borders of the ROI were defined by the extent of the MRSI slab in the z direction, the rostral border by the posterior part of the anterior cingulate cortex, and the posterior border by the postero-occipital fissure. Custom ROIs were generated by applying the following sequential steps to the anatomical ROIs of the PCP for each subject: (i) transformation to native space using the inverse normalization parameters M^{norm} ; (ii) multiplication with the tissue probability maps (probability for gray matter = 0.95; probability for white matter = 0.8); (iii) alignment with the MRSI slab using off-center parameters of the sMRI; (iv) binarization; and (v) re-gridding to the spectroscopic resolution. Binarization of voxels as either GM or WM was done before downsampling to MRSI resolution and rendering based on the respective tissue probability-thresholds was performed to avoid overlap between the GM and WM ROIs. All images were visually inspected to ensure accuracy of the registration and other processing steps.

2.5 MRSI reconstruction and processing

MRSI raw data were read into MATLAB using MRecon (Gyrotools Ltd, Winterthur, Switzerland). Prior to summing up spectra from individual MRSI voxels across the anatomical GM and WM ROIs the following processing steps have been performed: (i) Hamming filtering in both spatial directions; (ii) offcenter alignment between MRSI and MRI data caused by different spatial resolutions which lead to slightly different half-voxel-shifts and (iii) truncation of the FIDs after 512 of 2048 data points.

Custom written MATLAB routines were used for tailoring the above defined custom ROIs to tissue classes and anatomical labels, apply ROIs to the spectroscopic data for generating

1 averaged spectra from clustered MRSI voxels within gray matter and white matter sub-regions
2 of the PCP (**Figure 1A**). The aim of this procedure was to use very high spatial resolution MRSI
3 to cover the PCP sub-regions on the one hand, while avoiding the problems of small voxel sizes
4 (low SNR, low spectral quality) by separately clustering spectra from PCP gray matter and white
5 matter on the other hand. Thus, specific regional neurochemical profiles from gray matter only
6 versus white matter only could be obtained. In addition, the MRSI volumes used for computation
7 of the clustered MRSI spectra were quantified by counting the voxels-of-interest within the ROIs
8 of the PCP gray matter and white matter, and these voxel counts were used in the statistical
9 analysis to account for potential bias.

11 **2.6 Estimation of brain metabolites**

12 Average PCP gray and white matter spectra were analyzed using LC Model (Provencher, 1993),
13 and only metabolites with Cramér Rao lower bounds <20% from at least 25 subjects in all ROIs
14 were accepted. Moreover, data with insufficient data quality as indicated by aberrant linewidth
15 (full width at half-maximum, FWHM>1.31 ppm), SNR<4, or obvious artifacts were discarded
16 from the analysis. The LC Model basis set was simulated using the GAMMA library (Smith, et al.,
17 1994), and comprised the following 18 metabolites: NAA, mI, GABA, creatine (Cr), glycerophosphoryl-choline (GPC), phosphoryl-choline (PCh), glutamate (Glu), glutamine (Gln), glycine (Glc), ascorbate (Asc), myo-inositol (mI), aspartate (Asp), N-acetylaspartate-glutamate (NAAG), glutathione (GSH), scyllo-inositol (Scyllo), and. taurin (Tau). Phosphorylethanolamine (PE) was omitted from the basis set used for spectral fitting. Simulated macro-molecules were considered during the fitting (Henning, et al., 2009, Seeger, et al., 2003). tCho and Glx represent compound measures of GPC + PCh, and Glu + Gln, respectively. Cr-levels were used as an internal reference by normalizing all other metabolites to Cr as suggested earlier (Barker, et al., 2006, Near, 2014). The resulting normalized metabolite-to-creatine ratios were used for subsequent statistical analyses for group differences.

28 **2.7 Statistical analysis**

1 Statistical analysis was performed using Matlab (MathWorks, Natick, USA, Version: 8.4, R2014b),
2 its Statistics Toolbox (Version 9.1) and function for performing Intraclass Correlation Coefficient
3 (ICC) analysis (A. Salarian 2008, www.mathworks.com). A median split of the study sample was
4 used for separation of participants based on episodic memory performance: VLMT scores above
5 or equal to the group median were categorized as "high episodic memory performance", VLMT
6 scores below the median as "low episodic memory performance". For description of the sample,
7 demographic and volumetric data were presented using mean and standard deviation, and
8 compared for differences between the groups obtained by median-split. Non-parametric
9 Wilcoxon rank-sum was used to test the study population for a) differences in PCP NAA-levels
10 between gray and white matter; b) differences in PCP gray matter NAA-levels between VLMT
11 high and low-performers; c) differences in PCP white matter NAA-levels between VLMT high
12 and low-performers. The Holm-Bonferroni method was used to correct for multiple comparison
13 bias (Holm, 1979), and p-values <0.05 after adjustment were considered significant, p-values
14 <0.08 were considered as trend. Secondary analysis was performed for all other measurable
15 metabolites using the Wilcoxon rank-sum test to investigate group differences and the number
16 of testable metabolites as a factor for correction for multiplicity. For reduction of bias
17 introduced to metabolite-measures by potential variation of Cr as a reference, one-way random
18 single measures ICC analysis was performed to estimate chances for presence of a shared
19 confounding factor.

3. RESULTS

3.1 Characteristics of the study population

The study sample included 30 healthy elderly subjects (12 females, 18 males), with a mean age of 70 ± 5.7 years, mean duration of institutional education of 15.9 ± 2.4 years and mean MMSE of 29.4 ± 1 . Median VLMT performance was 12 out of a maximum score of 15 and used for dichotomizing the cohort into VLMT high and low-performers based on median-split. There were no group differences for age, education, or any cognitive task other than VLMT between VLMT high and low-performers. Assessment of the right and left hippocampus, left and right lateral ventricle and total gray matter provided no evidence for presence of significant differences in brain volumes between VLMT high and low-performers (**table 1**).

3.2 Quality control of metabolite measures as indicated by MRSI

Sufficient MRSI quality, as determined by visual inspection, Cramér Rao lower bounds $< 20\%$, as well as thresholds for linewidth and SNR, was achieved for NAA, tCho, mI, and Glx in 27 subjects (13 VLMT high-performers and 14 VLMT low-performers) and for GABA in 24 (10 VLMT high-performers and 14 VLMT low-performers) out of the 30 investigated subjects, respectively. No differences were observed for the sizes of the PCP ROIs indicated by the number of voxels between VLMT high and low-performers, confirming independence of neurometabolic group effects from the MRSI volumes-of-interest (**table 2**). ICC analysis did not provide evidence for a common confounding factor of the MRSI measures as possibly conferred by normalizing all measures to Cr: $r = -0.08$; $p = 0.96$; $F(28/145) = 0.56$.

3.3 Glx, tCho and mI levels significantly differ between PCP gray and white matter

When comparing NAA/Cr ratios between PCP gray and white matter, no significant difference could be observed (1.61 ± 0.32 , 1.54 ± 0.25 , $p = 0.37$, **table 2**). Secondary analysis provided evidence for significantly higher levels of Glx/Cr in PCP gray compared to white matter (1.75 ± 0.77 , 1.13 ± 0.29 , $p = 0.001$, significant after correction). Moreover, tCho/Cr and mI/Cr levels were significantly lower in PCP gray than in white matter (tCho: 0.31 ± 0.06 , 0.35 ± 0.05 ,

p=0.01, significant after correction; ml: 1.20 ± 0.24 , 1.35 ± 0.23 , $p=0.02$, significant after multiple comparison correction, **figure 2**).

3.4 Low episodic memory performance is associated with high gray matter NAA

When testing for group differences in NAA/Cr ratios between low and high VLMT performers, a significant association between low performance and higher levels of NAA was detected within PCP-gray matter (1.76 ± 0.33 , 1.45 ± 0.25 , $p=0.01$, significant after correction, **table 2** and **figure 3**). Secondary analysis of PCP gray matter metabolism resulted in nominally significant associations between low VLMT performance and higher levels of ml (1.29 ± 0.24 , 1.11 ± 0.20 , $p=0.04$, not significant after correction), Glx (2.06 ± 0.89 , 1.41 ± 0.37 , $p=0.03$, not significant after correction), and GABA (0.57 ± 0.27 , 0.36 ± 0.21 , $p=0.04$, not significant after correction) in VLMT low-performers compared to high-performers. There was no significant effect for VLMT performers in PCP white matter, as observable for any of the measurable metabolites (**table 2**).

4. DISCUSSION

High resolution ROI-based MRSI at 7 Tesla was used to investigate PCP neurometabolism and its potential association with low episodic memory performance in cognitively non-impaired elderly subjects. To our knowledge, this is the first report on increased PCP gray matter NAA in a context of low episodic memory performance. Our findings suggest an increase of neuronal mitochondrial energy capacity in relation to low episodic memory and, thus, may be consistent with earlier reports on increased PCP activity in populations at increased risk for AD (Cohen, et al., 2009, Filippini, et al., 2009, Mormino, et al., 2012, Sperling, et al., 2009). While several MRS-studies have investigated populations at risk for AD, to our knowledge ours is the first that distinctly allows for effects attributable to gray- versus white matter (Kantarci, et al., 2000, Metastasio, et al., 2006, Pilatus, et al., 2009, Riese, et al., 2015). Moreover, our findings are consistent with earlier reports on significant differences between gray- and white matter neurometabolism, as observed by high and ultra-high field strength MRSI (Bogner, et al., 2012, Gasparovic, et al., 2001, Henning, et al., 2009, Maudsley, et al., 2009).

Episodic memory dysfunction is a hallmark of AD (Albert, 2011), and low episodic memory performance in the non-demented elderly has been demonstrated to be associated with increased risk for AD (Elias, et al., 2000, Grober, et al., 2000, Linn, et al., 1995, Selkoe, 2013, B.J. Small, et al., 2000). In this context, particularly the development of markers capable of detecting decline from normal to subtly abnormal cognition is regarded to be a major challenge for the successful implementation of secondary-preventive clinical trials in AD (Sperling, et al., 2013). In the current study, episodic memory was measured by administering a delayed recall test for verbal memory (Helmstaedter, 2001, Helmstaedter and Durwen, 1990, Lezak, 1983, Müller, et al., 1997), which is consistent with neuropsychological testing performed in several earlier studies on sporadic AD and MCI (Albert, 2011, Gomar, et al., 2011, Grober, et al., 2000, Helmstaedter, 2001, Helmstaedter and Durwen, 1990, Lezak, 1983, Müller, et al., 1997). Participants in the current study had relatively high neuropsychological tests performance, however, there was significant variation within the normal range observable for episodic memory performance. When splitting the study population by median in high and low episodic memory performers,

hippocampal volumes as well as indicators of general brain atrophy did not differ between groups. This suggests that the variation in episodic memory present in the studied sample may be caused by metabolic change possibly preceding substantial neurodegenerative damage. Considering the well established association between low episodic memory and increased AD-risk, the absence of significant brain atrophy in our sample may furthermore be consistent with earlier reports on AD related brain alterations taking place decades before disease manifestation (Jack and Holtzman, 2013, Rajan, et al., 2015).

A sophisticated methodology was used for MRSI data acquisition at ultra-high field strength of 7 Tesla, including slice-selective FID acquisition (Henning, et al., 2009), optimized by an active B_0 shim-procedure (Fillmer, et al., 2015) (Kirchner, et al., 2015), and customized offline reconstruction. On the downside, part of these methodological efforts is required to compensate for inherent limitation of high magnetic field strength, including increased susceptibility to magnetic field inhomogeneity. While the so far established practice of using CRLB-thresholds of 20% has been criticized due to its potential bias by excluding measures of low concentration (Kreis, 2016), for the here investigated ROIs there were no spectra detectable that displayed both CRLB > 20% and nevertheless fulfilled general quality requirements for respective spectra. In the present study, metabolic maps of PCP gray- and white matter at high in-plane resolution of $3.5 \times 3.5 \text{ mm}^2$ could be generated for providing region-specific quantitative information for a total of six metabolic markers comprising NAA, tCho, mI, Glx, GABA as well as Cr. All metabolites were internally referenced to Cr to facilitate group comparisons and avoid bias introduced by individual variation of brain volumes and by the lack of an absolute quantification reference (Barker, et al., 2006, Near, 2014). By performing ICC analysis for all MRSI measures, no evidence for a significant confounding effect possibly exerted by variation of Cr as a reference, could be found. A particular advantage of the implemented MRSI sequence was the high spatial resolution allowing for assessment of gray versus white matter changes, as demonstrated to be a characteristic finding in brain pathology associated with aging and AD (Braak and Braak, 1991, Dickerson, et al., 2009, Schuff, et al., 2002, Serrano-Pozo, et al., 2011, Thal, et al., 2002). For mathematical reasons, binary masks were required to generate the averaged spectra from the

1 custom binary MRSI-ROIs, the nominal voxels do not fully represent actual VOI at 100%. While
2 the used GM and WM MRSI ROIs did not overlap, as observable by a "gap" of voxels between the
3 GM and WM ROIs in figure 1, lack of 100% independence between GM and WM MRSI measures
4 nevertheless cannot be completely ruled out. Our findings of general differences in tCho, mI and
5 Glx levels between PCP gray and white matter, as well as gray matter-specific NAA effects on
6 episodic memory performance, is consistent with earlier MRSI-studies and highlight the
7 relevance of tissue-specific neuro-metabolic analysis (Bogner, et al., 2012, Henning, et al.,
8 2009, Maudsley, et al., 2009).

9 NAA is metabolized in neuronal mitochondria from aspartic acid (Asp) and acetyl-coenzyme A
10 (Acetyl-CoA) and is highly abundant in the adult brain (Moffett, et al., 2007). It has been located in
11 neurons, oligodendrocytes, and myelin, where it is implicated in various biological processes
12 within the central nervous system, including mitochondrial energy production and peptidergic
13 neurotransmission (Clark, 1998, Moffett, et al., 2007). Consistent with its elementary role in
14 processes essential for cellular survival, there are several reports of decreased NAA levels in
15 neurodegenerative diseases characterized by progressive loss of brain tissue including clinically
16 manifest AD (Kantarci, et al., 2000, Klunk, et al., 1992) but also prodromal stages such as MCI
17 (Metastasio, et al., 2006, Pilatus, et al., 2009, Riese, et al., 2015), preclinical familial dementia
18 (Godbolt, et al., 2006, Kantarci, et al., 2010), or premanifest Huntington's disease (Unschuld, et
19 al., 2012). Studies on ischemic and focal brain damage suggest that NAA also reflects reversible
20 changes in neuronal health (Clark, 1998, Demougeot, et al., 2004, Gasparovic, et al., 2001).

21 The PCP region plays a central role in processing of memory tasks (Cavanna and Trimble,
22 2006, Miller, et al., 2008, Wagner, et al., 2005), and there are several studies that demonstrate
23 metabolic alterations and impaired functional properties of neuronal structures located within
24 the PCP region in neurodegenerative memory disorders including AD (Greicius, et al.,
25 2004, Minoshima, et al., 1997, Mosconi, 2005). Considering the fact that NAA synthesis is closely
26 linked to the ATP synthesis capacity of neuronal mitochondria (Clark, 1998, Maddock and
27 Buonocore, 2012, Moffett, et al., 2007), our finding of increased NAA being associated with low
28 episodic memory performance in cognitively healthy elderly subjects may reflect a

1 hypermetabolic state of the PCP gray matter. Thus, our results may be consistent with earlier
2 reports on increased gray matter metabolism in populations at risk for AD, particularly as
3 increased neuronal activity and metabolism has been demonstrated for the PCP-hub of the
4 default mode network in prodromal stages of AD (Cohen, et al., 2009,Filippini, et al.,
5 2009,Mormino, et al., 2012,Sperling, et al., 2009). The associations of high Glx, mI, and GABA
6 with low episodic memory performance did not remain significant after correction for multiple
7 testing and, thus, need to be interpreted with caution. However, as mI is considered to be a glial
8 marker of inflammation that has been reported to be increased in AD (Bitsch, et al., 1999,Miller,
9 et al., 1993), and Glx, and GABA reflect activity of major cerebral neurotransmitter systems that
10 also are affected by AD pathology (Gomar, et al., 2014,Kapogiannis and Mattson, 2011,Miller, et
11 al., 1993,Olson, et al., 2008,Riese, et al., 2015), our findings might indicate a general increase of
12 neuronal metabolic activity in a context of low episodic memory and thus be consistent with the
13 effects observed for NAA. The fact that metabolic change was not associated with hippocampal
14 or general brain atrophy may indicate potential reversibility due to absence of major
15 neurodegenerative damage. While the current study uses a cross-sectional design, additional
16 longitudinal studies are needed to investigate the temporal course of neurometabolic change in
17 elderly populations at increased risk for AD, as well as the impact of therapeutic interventions
18 aimed at aspartic acid metabolism.

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	Total sample	VLMT high- performers	VLMT low- performers	<i>p</i>
Sample, n (females; males)	30 (12; 18)	15 (7; 8)	15 (4; 11)	-
age (years)	70.0 (5.7)	69.2 (5.3)	70.7 (5.6)	<i>0.5</i>
education (years)	15.9 (2.4)	16.2 (2.6)	15.9 (2.2)	<i>0.87</i>
Mini Mental State (MMSE)	29.4 (1)	29.6 (0.5)	29.3 (0.9)	<i>0.56</i>
Verbal Learning & Memory (VLMT)	11.4 (3.2)	14.1 (0.8)	8.9 (2.6)	<i><0.001</i>
Boston Naming Test (BNT)	14.8 (0.4)	14.8 (0.4)	14.7 (0.46)	<i>0.5</i>
Digit Span forward	7.1 (1.5)	7.8 (1.3)	6.5 (1.6)	<i>0.076</i>
Digit Span backward	6.5 (1.6)	6.6 (1.5)	6.3 (1.7)	<i>0.314</i>
Trail Making Test (TMT-A/TMT-B)	2.4 (0.7)	2.6 (0.7)	2.2 (0.6)	<i>0.12</i>
Body Mass Index (BMI)	25.6 (3.7)	24.5 (2.3)	26.4 (4.5)	<i>0.534</i>
APOE-e4 carriers (n)	7	3	3	-
Hippocampal volume, right	2.6 (1.2)	2.3 (0.9)	2.8 (1.4)	<i>0.23</i>
Hippocampal volume, left	3 (1.6)	2.7 (0.9)	3.3 (2.2)	<i>0.35</i>
Lateral ventricle volume, right	1.9 (5)	1.9 (5.1)	1.9 (5)	<i>0.4</i>
Lateral ventricle volume, left	1.9 (6.1)	2 (5.8)	1.8 (6.4)	<i>0.88</i>
Total gray matter volume	5.5 (58.5)	5.6 (48.8)	5.5 (61.2)	<i>0.5</i>

Table 1: Demographics of the studied sample for high and low-episodic memory (median split based on delayed recall performance at VLMT), as indicated by mean and standard deviation. Hippocampal, ventricular and total gray matter volumes are indicated in ml.

	Total sample	VLMT high- performers	VLMT low- performers	<i>p</i>
A) PCP gray matter:				
NAA	1.61 (0.32)	1.45 (0.25)	1.76 (0.33)	* 0.01
tCho	0.31 (0.06)	0.28 (0.06)	0.33 (0.06)	0.06
mI	1.20 (0.24)	1.11 (0.20)	1.29 (0.24)	0.04
Glx	1.75 (0.77)	1.41 (0.37)	2.06 (0.89)	0.03
GABA	0.48 (0.26)	0.36 (0.21)	0.57 (0.27)	0.04
MRSI ROI (size)	67.64 (16.15)	66.62 (15.40)	68.53 (17.25)	0.61
B) PCP white matter:				
NAA	1.54 (0.25)	1.54 (0.27)	1.54 (0.23)	0.9
tCho	0.35 (0.05)	0.35 (0.04)	0.34 (0.06)	0.54
mI	1.35(0.23)	1.37 (0.27)	1.34 (0.19)	0.6
Glx	1.13 (0.29)	1.20 (0.36)	1.06 (0.23)	0.33
GABA	0.39 (0.24)	0.39 (0.20)	0.38 (0.28)	0.25
MRSI ROI (size)	88.57 (19.71)	85.71 (22.48)	91.43 (16.85)	0.32

Table 2: Metabolite levels within gray and white matter of the PCP region, as measured by 7 Tesla MRSI and analyzed using the LC Model software. All values were normalized to creatine, indicated are the resulting ratios for PCP gray (A) and white matter (B), respectively. Group differences significant after correction for multiple testing are indicated by "*". PCP=posterior cingulate and precuneus; VLMT=Verbal Learning Memory Test.

FIGURE CAPTIONS

Figure 1: Post-processing for ROI-based, tissue-specific metabolic analysis of the PCP region.

Axial view of post-processing. To derive estimates of gray and white matter metabolite levels from the PCP region (indicated by white dashed arrow), a semi-automated post-processing pipeline was established providing custom ROI of PCP gray and white matter corresponding to the resolution and field-of-view (FOV) of the high-resolution 7 Tesla MRSI. Figure 1 shows the sequential steps: 1) Gray and white matter maps were generated by segmentation of structural MRI using SPM segment. 2) A standard reference atlas (Hammers et al., 2003, Gaussias et al., 2008) was normalized to each individual's native space using inverse normalization based on the transformation parameters provided by SPM segment. 3) The PCP-ROI, defined on the reference atlas, is shown on a standard MNI template brain (red color: precuneus; green color: posterior cingulate; the yellow line indicates the position of the MRSI slab above the corpus callosum covering the PCP region. 4) Tissue-specific ROIs of PCP gray and white matter were derived by multiplication of the tissue-probability maps with the PCP-ROI, which are shown in T1 and MRSI resolution for demonstration purposes (1: T1-resolution, red color indicates gray matter map, blue color indicates white matter map; 4: after re-gridding of the tissue probability maps to MRSI-resolution). The resulting ROIs of PCP gray and white matter were aligned with the FOV of the MRSI slab using off-center parameters and re-gridded to the spatial resolution of the MRSI (3.5x3.5). 5) Custom ROIs of PCP gray and white matter aligned with the MRSI grid. 6) MRSI voxels covering gray (red color) and white matter (blue color) of the PCP were used to create average tissue-weighted MRSI spectra that were analyzed by LCModel.

Figure 2: Average metabolite levels within gray and white matter of the PCP, as obtained by MRSI at 7 Tesla and normalization to Cr. Significance after correction for multiple testing is indicated by "*".

Figure 3: Average gray matter metabolite levels within the PCP of individuals below and above the median of VLMT scores measured within the studied sample. Metabolites were normalized to Cr, significance after correction for multiple testing is indicated by "*".

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