Published in final edited form as:

J Neuroimaging. 2017 November ; 27(6): 630–636. doi:10.1111/jon.12454.

Decreased glutamate levels in patients with amnesic mild cognitive impairment: an sLASER proton MR spectroscopy and PiB-PET study

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Abstract

Background and purpose—Glutamate levels may be informative about the declining neuronal health in the central nervous system. We used an advanced proton MR spectroscopy (¹H-MRS) protocol comprised of semi-localization by adiabatic selective refocusing (sLASER) localization and FAST(EST)MAP shimming for detection of alterations in brain glutamate concentrations in patients with amnesic mild cognitive impairment.

Methods—Participants with amnesic mild cognitive impairment (n=14; median age=80) and age- and sex-matched clinically normal controls (n=32; median age=79) from the population-based Mayo Clinic Study of Aging were recruited prospectively to the 3T single-voxel ¹H-MRS study that examined metabolite changes in the posterior cingulate gyri. To be included, controls had to have low β -amyloid load on [¹¹C] Pittsburgh Compound B (PiB)-PET (standard uptake value ratio; SUVR <1.42) and patients with amnesic mild cognitive impairment had to have high β -amyloid load (SUVR $\geq$ 1.42).

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All authors have read the final version of the manuscript and agreed with it.

Disclosure

Dr. B. Zeydan, Dr. D. Deelchand, Ms. N. Tosakulwong, Dr. O. Kantarci, Dr. M. Machulda and Mr. T. Lesnick: Nothing to disclose.

Results—Glutamate concentration and the glutamate/*myo*-inositol ratio were lower in patients with amnesic mild cognitive impairment than clinically normal controls ($p<0.05$). Higher global cortical PiB-PET SUVR correlated with lower glutamate/*myo*-inositol ($r=-0.3$, $p=0.04$).

Conclusions—The advanced sLASER with FAST(EST)MAP shimming is a promising protocol for identifying glutamate alterations. Advanced ^1H -MRS protocols may add to the understanding of early Alzheimer's disease pathophysiology through detection of glutamate concentration in posterior cingulate gyri of individuals with amnesic mild cognitive impairment.

Keywords

magnetic resonance spectroscopy; amnesic mild cognitive impairment; glutamate; positron emission tomography

Introduction

Biomarkers of Alzheimer disease (AD)-associated neurodegeneration are critical for early diagnosis, for tracking early disease progression and identifying individuals for potential preventive interventions. Furthermore, such biomarkers may provide a window into the mechanisms of neurodegeneration in AD.

Glutamate homeostasis is accomplished by the neuron-astrocyte interaction and glutamate concentration is altered by neuronal density and neurodegeneration.^{1, 2} Therefore a decrease in glutamate levels in the central nervous system would be a potential biomarker of declining neuronal health. We hypothesized that decreased glutamate levels can be observed in the posterior cingulate gyrus of patients with amnesic mild cognitive impairment (aMCI) compared to controls as an early biomarker of neurodegeneration.

Proton magnetic resonance spectroscopy (^1H -MRS) detects neurochemicals that are associated with neuronal or synaptic integrity, glial activation, and the pathologic stage of AD.³⁻⁵ These neurochemical alterations are detected as early as the prodromal stage of AD, such as in patients with aMCI.⁶ For example, elevation of *myo*-inositol can be detected in patients with aMCI, before the decrease in the neuronal integrity biomarker *N*-acetylaspartate (NAA) in patients with AD.⁷ In contrast to metabolites that are consistently abnormal in AD such as NAA and *myo*-inositol,⁸ little is known about glutamate, a metabolite excluded in most of the aMCI studies because of the methodological difficulties in separating glutamate from the glutamine signal using conventional acquisition protocols. However, reliable separation of glutamate and glutamine in ^1H -MRS strongly depends on both the spectral data quality and the data analysis methods.

The conventional full intensity ^1H -MRS protocols are hampered by large chemical shift displacement errors (CSDE) at 3T.⁹ In contrast, the semi localization by adiabatic selective refocusing (sLASER) methodology achieves more accurate localization with minimal CSDE.^{10, 11} Importantly, an optimized sLASER protocol that incorporates variable pulse power optimized relaxation (VAPOR) water suppression, outer volume suppression, voxel based B_0 and B_1 calibrations and FAST(EST)MAP shimming, enables highly reproducible metabolite quantification at 3T, with between-session coefficients of variance (CV) 5% for

the 5 major neurochemicals, including glutamate.¹² sLASER protocol's advanced features improve the spectral quality through superior water suppression, minimization of unwanted coherences and negligible CSDE at short echo-times. The FAST(EST)MAP shimming,¹³ utilized for localized shimming together with the sLASER protocol, further contributes to the improvement in the metabolite quantification, by providing narrower spectral linewidths.

The conventional ¹H-MRS protocols were commonly used in previous studies in patients with aMCI.^{7, 14–23} However, findings regarding glutamate alterations in patients with aMCI have been inconsistent using conventional ¹H-MRS acquisitions, and glutamate has been reported as a sum with the metabolite glutamine (Glx).^{24–27} An advanced sLASER protocol may provide reliable quantification of glutamate and identify early glutamate alterations in patients with aMCI.

In this study, an advanced sLASER MRS protocol was used in the clinical setting to test the feasibility of detecting glutamate and to determine whether glutamate levels measured by the advanced sLASER protocol are decreased in patients with aMCI.

Methods

Participants

Consecutive participants with aMCI using the most recent diagnostic criteria⁶ (n=14) and age- and sex-frequency matched clinically normal controls (CN; n=32) from the population-based Mayo Clinic Study of Aging²⁸ were prospectively recruited to this study.

In addition to clinical evaluations and neuropsychological testing; to make sure we captured the lowest risk for AD group, controls had to have low β -amyloid load on [¹¹C] Pittsburgh Compound B (PiB)-PET (standard uptake value ratio; SUVR <1.42). To capture the highest risk for AD group, patients with aMCI had to have high β -amyloid load (SUVR $\geq$ 1.42)²⁹ on their most recent PiB-PET scan that was performed on average within 15 months of the ¹H-MRS examination. Individuals who had a neurological disorder such as a brain tumor, epilepsy, multiple sclerosis or stroke that would have an effect on ¹H-MRS measurements were not invited to participate in this study.

Participants were enrolled after giving informed consent and the approvals were obtained from the Institutional Review Board.

MRI and ¹H-MRS Acquisitions

All participants underwent the ¹H-MRS acquisition scheme in one scanning session: the advanced modified sLASER protocol with FAST(EST)MAP B₀ shimming.¹⁰ The study was conducted by 3 rotating clinical MRI technologists on a 3T Verio scanner (Siemens Medical Solutions, Erlangen, Germany), operating with the VB17 software. A 32-channel phased array head coil was used for signal reception.

The ¹H-MRS voxel included the posterior cingulate gyri bilaterally (Figure-1). In order to select an 8mL of volume-of-interest in the posterior cingulate gyrus, a 3D T₁-weighted MPAGE sequence ($T_R/T_E=2300/2.98$ ms, spatial resolution=1×1×1.2 mm³) was

performed. The posterior cingulate cortex is an important hub for resting-state connectivity networks,³⁰ it is affected by AD's β -amyloid pathology³¹ and shows high PiB uptake.³² The posterior cingulate cortex is also affected by neurofibrillary tangle tau pathology of AD, relatively earlier than other neocortical regions in the disease course.³³ Furthermore, the posterior cingulate cortex is a region of reduced glucose metabolism in cognitively normal individuals with a genetic risk of AD.³⁴

A single-voxel proton spectrum for each individual was acquired in the same session using sLASER¹⁰ sequence with VAPOR water suppression ($T_R/T_E=5000/28$ ms, 64 averages) and FAST(EST)MAP shim.¹³ In addition, unsuppressed water signals were acquired for eddy current correction and for absolute quantification, as described previously.³⁵

Metabolite Quantification

Participants were scanned at the Mayo Clinic. The raw de-identified ¹H-MRS data were transferred to the University of Minnesota for post-processing using Matlab. Eddy current corrected spectra were quantified with LCModel 6.3-0G³⁶ using water scaling.³⁵

Previously published chemical shifts and J -coupling values were utilized to simulate basis sets with density-matrix formalism.^{37, 38} Non-localized simulation with actual radiofrequency shapes and timings was used, due to the low CSDE. To determine the CSF contribution in the volume-of-interest, twelve T_{ES} (28–4000 ms) were utilized to acquire fully relaxed unsuppressed water signals with the sLASER sequence^{35, 39} and concentrations of all metabolites were quantified after correcting for the CSF content in the voxel.

Metabolites that had Cramér-Rao lower bounds (CRLBs) $\geq 20\%$ in at least 50% of the acquired spectra were selected for analysis.⁴⁰ If the correlation coefficient between two metabolites was <-0.7 , then the sum of the metabolites was used; such that total creatine (Cr) included creatine and phosphocreatine, total choline (Cho) included glycerophosphocholine and phosphocholine and total N -acetylaspartate (NAA) included N -acetyl-aspartate and N -acetyl-aspartylglutamate. Because *myo*-inositol elevation is expected in patients with aMCI, we also investigated glutamate/*myo*-inositol ratio.

PiB-PET

A PET/CT scanner (DRX; GE Healthcare) operating in 3-dimensional mode was used for PiB-PET imaging. After the subjects were injected with PiB (292–729 MBq) and following a 40-minute uptake period, a 20-minute PiB scan was obtained. Four 5-minute dynamic frames at 40 to 60 minutes post-injection were acquired. As previously described, PET analysis was performed using an automated image processing pipeline.⁴¹ The bilateral parietal, temporal, prefrontal, orbitofrontal and anterior cingulate GM regions referenced to cerebellar gray matter were used to obtain the global cortical PiB retention SUVR.⁴¹ A global cortical-to-cerebellar ratio cut-off point of 1.42 was obtained to classify participants as having high β -amyloid load.²⁹ This specific cut-off point was defined by the reliable worsening cut-point method, which distinguishes individuals as having high or low β -amyloid load.²⁹

Statistical analysis

Group differences were assessed using Fisher's Exact or Wilcoxon Rank Sum tests. We used Spearman correlations to measure associations between PiB-PET SUVrs and metabolite concentrations or ratios quantified by sLASER in the entire cohort and in the CN and the aMCI groups separately. Area under receiver operating characteristic curve (AUROC) analysis was carried out to measure the ability of the sLASER protocol to distinguish the two clinical groups using ^1H -MRS metabolite concentrations and ratios.

Results

A total of 46 individuals who were either CN (n=32) or aMCI (n=14) were recruited to the study (Table-1). Age, sex or time between PiB-PET and MRI/ ^1H -MRS did not differ between groups. As expected, the Mini-Mental State Examination scores were lower ($p<0.001$) and PiB-PET SUVrs and the frequency of *APOE* $\epsilon 4$ carriers were higher in the aMCI group than the CN group ($p<0.01$).

Glutamate concentration and the glutamate/*myo*-inositol ratio were lower in the aMCI group than the CN group using the sLASER protocol ($p<0.05$). Creatine, *myo*-inositol, choline and *N*-Acetyl-Aspartate concentrations did not differ between patients with aMCI and CNs ($p>0.05$) (Table-2). The boxplots in Figure-2 show the distribution of individual participant's values. The AUROC estimates were used to distinguish patients with aMCI and CNs for each metabolite concentration and ratio. Glutamate concentration could separate aMCI and CN groups with an AUROC estimate of 0.70 (95% confidence interval: 0.51–0.85) and the glutamate/*myo*-inositol ratio with an AUROC estimate of 0.71 (95% confidence interval: 0.54–0.86) (Figure-3).

There was a negative correlation between the global cortical PiB-PET SUVr and glutamate/*myo*-inositol ratio in the entire cohort of 46 participants ($r=-0.3$, $p=0.04$). However, there were no significant correlations between PiB-PET SUVr and metabolite levels or ratios when CNs and patients with aMCI were investigated separately. Figure-4 displays example ^1H -MR spectra and PiB-PET of a CN individual and a patient with aMCI.

Discussion

In this study, we observed that glutamate concentration and glutamate/*myo*-inositol ratio were lower in patients with aMCI, compared to the CN individuals. Furthermore, glutamate/*myo*-inositol ratios measured by sLASER inversely correlated with global cortical PiB-PET SUVr.

Patients with aMCI who have high β -amyloid deposition on PiB-PET are at an increased risk of progressing to AD dementia than patients with aMCI, who do not have elevated levels of β -amyloid.^{6, 42, 43} We studied the patients with aMCI with high β -amyloid load to investigate the accuracy of advanced sLASER protocol in distinguishing patients with aMCI from CN individuals who had low β -amyloid deposition on PiB-PET. In addition to glutamate levels, we also evaluated the glutamate/*myo*-inositol ratio in order to increase the diagnostic sensitivity, because *myo*-inositol levels tend to be higher in the aMCI group

compared to the CN group. We were unable to study glutamine levels separately, since glutamine did not have CRLBs 20% in at least 50% of the acquired spectra.

Glutamate is the primary excitatory neurotransmitter of the CNS. After being released from the neuron into the synapse, it is removed by the astrocytes, where it is transformed into glutamine and sent back to the neuron to be re-used as glutamate. As such, glutamate is a marker for the health of neuron-astrocyte interaction and contributes to mechanisms of neuronal function, plasticity, metabolism, as well as excitotoxicity.^{1, 2} Excessive and prolonged excitatory glutamatergic stimulation leads to neuronal cell death,^{44, 45} which is a feature of neurodegeneration observed in AD. Neurodegeneration leads to a reduction in glutamate levels due to loss of neuronal viability and synaptic activity.

¹H-MRS typically detects the combined peaks of glutamate plus glutamine (Glx), since it is difficult to separate them by conventional protocols. Hence, the previously reported results of reduced Glx in patients with aMCI and AD may be due to glutamate or glutamine or both. The advanced sLASER protocol enabled detection of a glutamate reduction in patients with aMCI, which was not demonstrated with conventional PRESS protocols in patients with aMCI.^{24–27, 46}

Previous studies using conventional protocols have reported reduced concentrations of Glx in patients with aMCI²⁴ and AD dementia^{46–48} or normal Glx levels in patients with aMCI.^{25–27, 46} Using sLASER acquisition from the hippocampus, decreased glutamate concentration was demonstrated in patients with AD dementia, but not in patients with aMCI.⁴⁹ In the current study, we were able to detect decreased glutamate levels in the aMCI group compared to the CN group in the posterior cingulate gyrus, using the advanced sLASER protocol. This was particularly noteworthy considering the small sample size. The reasons for the discrepant findings may be twofold: 1) the hippocampus, being close to the tissue-air-bone-CSF interface at the skull base, is a difficult region for shimming. In contrast, the posterior cingulate gyrus is not close to the skull base, which may improve the detection of metabolite alterations; 2) there may be regional differences in glutamate reduction in AD, affecting the posterior cingulate cortex more significantly than the hippocampus. For example β -amyloid deposition on PiB-PET is significantly higher in the posterior cingulate cortex, compared to the medial temporal lobe in AD.⁵⁰ In fact, we observed a correlation between global cortical PiB-PET SUVR and the glutamate/*myo*-inositol ratio. This correlation may also be driven by the patients with aMCI who had higher PiB-PET SUVR values as one of the study inclusion criteria and had lower glutamate/*myo*-inositol ratios.

Earlier ¹H-MRS studies in patients with aMCI, conducted using the conventional protocols, such as PRESS, showed that the ¹H-MRS abnormalities identified in patients with aMCI were similar, but only milder compared to the abnormalities identified in patients with AD dementia.^{8, 14, 16, 18, 21, 25, 51–63} In patients with aMCI, the elevation in the *myo*-inositol level was shown to precede the decrease in the NAA level.^{7, 21, 63} Longitudinal alterations in ¹H-MRS metabolites and the relationship between metabolite concentrations and cognition in patients with aMCI and AD dementia were also demonstrated.^{15, 17, 19, 20, 22, 23, 26, 27, 46, 63–71}

Although the *myo*-inositol concentration was relatively higher in the aMCI group than the CN group in the current study, the differences between groups were not statistically significant. This is likely due to the smaller sample size, a limitation of the current study, relative to prior studies that reported *myo*-inositol elevation in patients with aMCI.^{15, 19, 22, 26, 27, 72} We also did not find a difference in total NAA levels between groups, which is in agreement with previous findings that an alteration in the total NAA level is not typically observed until patients with aMCI progress to AD dementia.²⁰ It is possible that, if brain regions other than the posterior cingulate gyrus are studied, our findings may change. For example, reduced glutamate levels were not observed in the hippocampus of patients with aMCI compared to controls.⁴⁹

Glutamate neurotransmission and glutamate-glutamine cycling alterations play an important role in AD-related neuronal damage.⁷³ The advanced sLASER protocol can be implemented in the clinical setting, and through reliable quantification of glutamate, may contribute to the understanding of pathophysiologic mechanisms of early neurodegeneration in AD. Although our findings are from the posterior cingulate cortex, depending on the stage of the neurodegeneration associated with AD pathophysiology, findings may change over time in other regions of the brain. Future research on the longitudinal outcomes of patients with aMCI with glutamate reduction and monitoring the longitudinal change in glutamate levels may contribute to the understanding of early disease mechanisms in patients with aMCI.

Acknowledgments

We would like to thank the MR technologists; Vicki L. Knudsen R.T., Brian Rucker R.T. and Suzanne L. Carlson R.T. who contributed to the study by scanning the participants.

This study was funded by the Minnesota Partnership for Biotechnology and Medical Genomics, the NIH [R01 AG040042, P50 AG016574, U01 AG006786, C06 RR018898, R01 NS080816], The Elsie and Marvin Dekelboum Family Foundation and the Robert H. and Clarice Smith and Abigail Van Buren Alzheimer's Disease Research Program. The Center for Magnetic Resonance Research is supported by the National Institute of Biomedical Imaging and Bioengineering (NIBIB) grant P41 EB015894 and the Institutional Center Cores for Advance Neuroimaging award P30 NS076408. The funding sources had no role in study design, collection, analysis, interpretation, or decision to submit this paper. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Dr. V. Lowe: Reports grants from NIH, grants from Liston Foundation, grants from The Elsie and Marvin Dekelboum Family Foundation, during the conduct of the study; grants from GE Health Care, grants from Siemens Molecular Imaging, grants from AVID Radiopharmaceuticals, personal fees from Piramal Imaging and Merck Research, outside the submitted work.

Dr. D. Knopman: Serves on a Data Safety Monitoring Board for Lundbeck Pharmaceuticals and for the Dominantly Inherited Alzheimer's Disease Treatment Unit. He is participating in clinical trials sponsored by Lilly Pharmaceuticals and Tau Rx Pharmaceuticals. He receives research support from the NIH.

Dr. R. Petersen: Chaired a Data Monitoring Committee of Pfizer, Inc. and Janssen Alzheimer Immunotherapy and serves as a consultant for Hoffman La Roche, Inc., Merck, Inc., Genentech, Inc., Biogen, Inc., Eli Lilly and Co. and receives research support from the NIH (P50 AG016574 [PI] and U01 AG006786 [PI], R01 AG011378 [Co-I], and U01 AG024904 [Co-I]).

Dr. C. Jack: Reports consulting services for Eli Lilly Co, funding from the NIH (R01 AG011378, U01 AG024904, R01 AG041851, R01 AG037551, R01 AG043392, and U01 AG006786) and research support from the Alexander Family Professorship of Alzheimer's Disease Research.

Dr. G. Öz: Reports grant support from the NIH, Bob Allison Ataxia Research Center, Parkinson's UK, CHDI Foundation, Inc and Friedreich's Ataxia Research Alliance during the conduct of the study.

Dr. K. Kantarci: Serves on the data safety monitoring board for Pfizer Inc. and Janssen Alzheimer's Immunotherapy Takeda Global Research & Development Center, Inc.; and she is funded by the NIH.

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Figure-1.

A. The posterior cingulate gyrus voxel on a mid-sagittal T1-weighted image.

Figure-2.

Boxplots show glutamate and *myo*-inositol concentrations (mM) and glutamate/*myo*-inositol ratio in the CN and aMCI groups.

CN = clinically normal, aMCI = amnesic mild cognitive impairment

Figure-3.

AUROC estimates (95% CI) of metabolite concentrations and glutamate/*myo*-inositol ratio

AUROC = Area under receiver operating characteristic curve

Decreased Glutamate Levels in Patients with Amnesic Mild Cognitive Impairment: An sLASER Proton MR Spectroscopy and PiB-PET Study

Figure-4.

Example ¹H-MR spectra and PiB-PET of **A.** a clinically normal individual (glutamate concentration = 10.17 mM and PiB-PET SUVR = 1.24) and **B.** a patient with amnesic mild cognitive impairment (glutamate concentration = 7.40 mM and PiB-PET SUVR = 2.90). Cr = total Creatine (=creatine + phosphocreatine), Ins = *myo*-inositol, Cho= total choline (=glycerophosphocholine + phosphocholine), Glu = glutamate, Gln = glutamine, NAA = total *N*-acetyl-aspartate (=N-acetyl-aspartate + *N*-acetyl-aspartylglutamate)

Table 1

Characteristics of participants

	CN (n=32)	aMCI (n=14)	<i>p</i>
Number of women	16 (50%)	5 (36%)	0.52
<i>APOE</i> ε4 carrier	8 (25%)	10 (71%)	0.007
Age at MRI, years	79 [74, 83]	80 [76, 86]	0.27
Mini-Mental State Examination score	28 [28, 29]	26 [24, 27]	<0.001
PiB-PET SUVRs	1.32 [1.29, 1.34]	2.15 [2.01, 2.39]	<0.001
Time between MRI and PiB-PET, years	1.30 [1.19, 1.45]	1.26 [0.45, 1.54]	0.48

Data shown except sex are median [interquartile range].

P-values were assessed using Fisher's Exact or Wilcoxon Rank Sum tests.

CN = clinically normal, aMCI = amnesic mild cognitive impairment, PiB = Pittsburgh compound B, SUVR = standard uptake value ratio.

Table 2

Metabolite levels in CN individuals and patients with aMCI.

Metabolite or ratio	sLASER ¹ H-MRS		
	CN (n=32)	aMCI (n=14)	<i>p</i>
Creatine * (mM)	8.7 [8.0, 9.0] (7.5, 12.1)	8.0 [7.7, 8.7] (7.5, 13.1)	0.13
<i>Myo</i> -Inositol (mM)	6.9 [6.3, 7.7] (5.5, 10.4)	7.1 [6.4, 8.2] (5.4, 11.6)	0.47
Choline * (mM)	1.8 [1.7, 2.1] (1.3, 3.6)	1.8 [1.7, 2.0] (1.1, 2.6)	0.77
Glutamate (mM)	8.9 [8.4, 9.2] (6.7, 11.2)	8.1 [7.8, 8.7] (7.0, 12.6)	0.04
<i>N</i> -Acetyl-Aspartate * (mM)	10.8 [10.3, 11.6] (9.7, 14.0)	10.4 [10.1, 11.5] (9.1, 14.4)	0.31
Glutamate/ <i>Myo</i> -Inositol	1.31 [1.19, 1.43] (0.91, 1.59)	1.10 [1.04, 1.25] (0.83, 1.39)	0.02

Data shown are median [interquartile range] (range). P-values were assessed using Wilcoxon Rank Sum test.

* Creatine = creatine + phosphocreatine; Choline = glycerophosphocholine + phosphocholine; *N*-acetyl-aspartate = *N*-acetyl-aspartate + *N*-acetyl-aspartylglutamate.

CN = clinically normal, aMCI = amnesic mild cognitive impairment, sLASER = semi localization by adiabatic selective refocusing