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Prefrontal GABA Levels Correlate with Memory in Older Adults at High Risk for Alzheimer's Disease

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Abstract

γ-Aminobutyric acid (GABA), a primary inhibitory neurotransmitter in the brain, plays a significant role in aging and in neurodegenerative disorders, including Alzheimer's disease (AD). We investigated the relationship between GABA levels in the dorsomedial/dorsoanterolateral prefrontal cortex (DM/DA-PFC) and memory in high-AD risk participants. Thirty-eight participants (14 Cognitively Normal [CN], 11 with Subjective Cognitive Decline (SCD), and 13 Mild Cognitive Impairment [MCI]) underwent magnetic resonance spectroscopy at 3 Tesla. SCD and MCI participants were grouped together to form a single high-AD risk group ($N = 24$) for the purposes of statistical analyses. Partial correlations of GABA+/Cr level with verbal memory, assessed on California Verbal Learning Test-II, and nonverbal memory, assessed on Brief Visuospatial Memory Test and Rey-Osterrieth test, were examined separately within the high-AD risk and CN groups. GABA+/Cr levels were positively correlated with long-delayed verbal memory ($r = 0.69$, $P = 0.009$) and immediate nonverbal memory ($r = 0.97$, $P = 0.03$) in high-AD risk, but not in CN participants. These results remained significant after controlling for depression. These preliminary findings, which require replication due to the limited sample sizes, are the first report of an association between GABA+/Cr levels within the DM/DA-PFC and memory performance in high-AD risk individuals.

Key words: γ-aminobutyric acid, cognition, magnetic resonance spectroscopy, nonverbal memory, verbal memory

Introduction

Alzheimer's disease (AD) is the most common age-related neurodegenerative disorder, affecting ∼24 million of the elderly population globally (Mayeux and Stern 2012). Around 1.3 million older adults over the age of 65 in the United States of America are projected to be diagnosed with AD by 2050 (Hebert et al. 2013). AD-related changes in the brain may occur 10–20 years prior to the diagnosis; thus, it is critical that we identify biomarkers for AD in its earliest stages (Jack et al. 2010).

Animal models of AD suggest that synaptic dysfunction due to the accumulation of amyloid β (Aβ) oligomers precedes

neuronal loss (Selkoe 2002; Palop and Mucke 2010). Of the 2 major types of synapses in the central nervous system, glutamatergic and γ-aminobutyric acid (GABA)-ergic, which generate excitatory and inhibitory outputs, respectively, there is abundant evidence of impaired glutamatergic system in the pathogenesis of AD (Benarroch 2018). However, emerging data also support the contribution of GABAergic remodeling in the early stages of AD progression (Calvo-Flores Guzmán et al. 2018).

GABA is widely distributed in the brain and binds to ionotropic or metabotropic receptors on postsynaptic neurons to inhibit their function (Govindpani et al. 2017). As the primary inhibitory neurotransmitter in the brain, GABA plays a critical role in regulating responsiveness and excitability within neural networks and in synchronizing cortical neuronal signaling activity (Govindpani et al. 2017). Thus, GABA is involved in a wide range of physiological and biochemical processes, including the regulation of cognition, memory and learning, neural development, and adult neurogenesis (Govindpani et al. 2017). While GABAergic dysfunction has long been implicated in neuropsychiatric conditions such as epilepsy, mood disorders, autism, and schizophrenia (Verhoeff et al. 1999; Luscher et al. 2011; Fatemi and Folsom 2015), alterations of GABAergic circuits, whether as a primary event or as a compensatory response to excitotoxicity, also appear to contribute to AD progression by disrupting overall network function (Sailasuta et al. 2012; Huang et al. 2017). The GABAergic system has been recently identified as a potential target for AD intervention (Luo et al. 2015; Li et al. 2016; Govindpani et al. 2017). Preclinical studies suggest that GABA-A receptor agonists may have a neuroprotective effect, while antagonists enhance cognition, but these findings need to be corroborated using large clinical trials (Calvo-Flores Guzmán et al. 2018).

Other data show that cellular expression of GABA receptors may also be altered in AD. While there is an overall trend toward enhanced expression of the GABA-A receptor in the CA3 region, the findings are more complex within the CA1 region, with increased cellular expression in some subunits and decreased expression in others (Kwakowsky et al. 2018). Relevant to the current study, GABA clearance is also impaired in the AD brain (Fuhrer et al. 2017).

In addition, there is evidence of reduced GABA within temporal, medial frontal and parietal cortices in AD (Gueli and Taibi 2013; Bai et al. 2015). GABA level within the frontal lobes also decreases with age, cognition, and cortical volume (Porges, Woods, Edden, et al. 2017a). Furthermore, a study of healthy aging showed an association between GABA level within the dorsal anterior cingulate and executive functions (Marenco et al. 2018), suggesting that GABA within the dorsal anterior cingulate may be particularly sensitive to cognitive ability. This region overlaps with the prefrontal regions that show reduced GABA in major depressive disorder, which include the dorsomedial prefrontal cortex (dmPFC) in addition to dorsal anterior cingulate cortex (Hasler et al. 2007). Executive dysfunction and neuropsychiatric symptoms such as depression and anxiety may be early changes in AD (Geda et al. 2006; Guild et al. 2014; Mah et al. 2015). Taken together, these findings suggest that GABA level within the dorsomedial and dorsoanterolateral PFC regions may be associated with both cognition and mood.

In the current study, we measured GABA levels relative to Creatine (Cr) (GABA+/Cr) within the dorsomedial/dorsoanterolateral prefrontal cortex (DM/DA-PFC) in older adults at risk for developing AD and in a cognitively normal (CN) group using Magnetic Resonance Spectroscopy (MRS). The high-AD risk group included older adults with mild cognitive impairment (MCI), a prodrome of AD (Petersen 2004), and older adults with subjective

cognitive decline (SCD), currently conceptualized as a preclinical stage of AD (Jessen et al. 2014). We assessed the relationship between DM/DA-PFC GABA+/Cr and memory within the high-AD risk group and within the CN group. We hypothesized that GABA+/Cr would be positively associated with verbal and non-verbal memory in high-AD risk participants, with weaker relationships in the CN group.

Materials and Methods

Participants

62 older adults (27 CN, 18 SCD, and 17 MCI) were scanned using MRS. Data from 24 participants (13 CN, 7 SCD, and 4 MCI) were excluded from the final analysis due to inability to obtain post-processing fitting or poor spectral quality of MRS data due to movement artifacts. Thus, the final sample included 38 participants, which consisted of 14 CN (9 females, mean age = 68.3 years, SD = 6.4); 11 SCD (6 females, mean age = 75.1, SD = 6.6); and 13 MCI (5 females, mean age = 74.3, SD = 7.4). Due to their small sample sizes, SCD and MCI participants were grouped together to form a single group of high-AD risk participants (N = 24) for the purposes of statistical analyses.

All participants were aged 60 years or older with memory concerns, English language proficiency adequate for neuropsychological assessment, free of neurologic or psychiatric illnesses that may account for memory problems (e.g., previous stroke, Parkinson's disease, depression, and dementia), and free of current psychotropic or memory enhancing medications within the past 3 weeks. Both CN and SCD participants scored within the normal range for age and gender on a neuropsychological battery. SCD participants had subjective memory concerns as defined by their responses on the self-report Memory Functioning Questionnaire- Frequency of Forgetting subscale (MFQ-FF) (Gilewski et al. 1990), a measure of subjective memory ability. Thus, <u>lower</u> scores on the MFQ-FF reflect subjective memory concerns. The MFQ-FF was selected as a measure of subjective memory concern because lower scores are sensitive to Aβ burden on Positron Emission Tomography imaging (Chételat et al. 2013). SCD participants' MFQ-FF scores fell below the median score of 182 in our larger study sample of 143 participants, while CN participants scored above the median of 182 on the MFQ-FF. MCI participants met the National Institute on Aging and Alzheimer's Association criteria for MCI (Albert et al. 2011) as well as specific neuropsychological criteria of performance on 2 or more memory tests greater than 1.5 SD lower than expected relative to overall intellect (Troyer et al. 2016). The study was approved by the Research Ethics Board of Baycrest Health Sciences.

Data Acquisition

We utilized the noninvasive J-difference edited MEscher-GArwood-Point Resolved Spectroscopy (MEGA-PRESS) pulse sequence to measure GABA level (Mescher et al. 1998), which allows fitting of GABA+ signals despite the presence of stronger signals of overlapping metabolites (Mullins et al. 2014). Proton MRS spectra were acquired from our region of interest (ROI) DM/DA-PFC which extended a $25 \times 40 \times 25$ mm^3 voxel and included portions of Brodmann areas 9, 24, and 32, dorsal and pregenual anterior cingulate gyrus, and adjacent medial frontal gyrus (Hasler et al. 2007). This ROI was selected based on a study that showed reduced GABA within DM/DA-PFC in patients with major depressive disorder (Hasler et al. 2007). Figure 1*a* shows
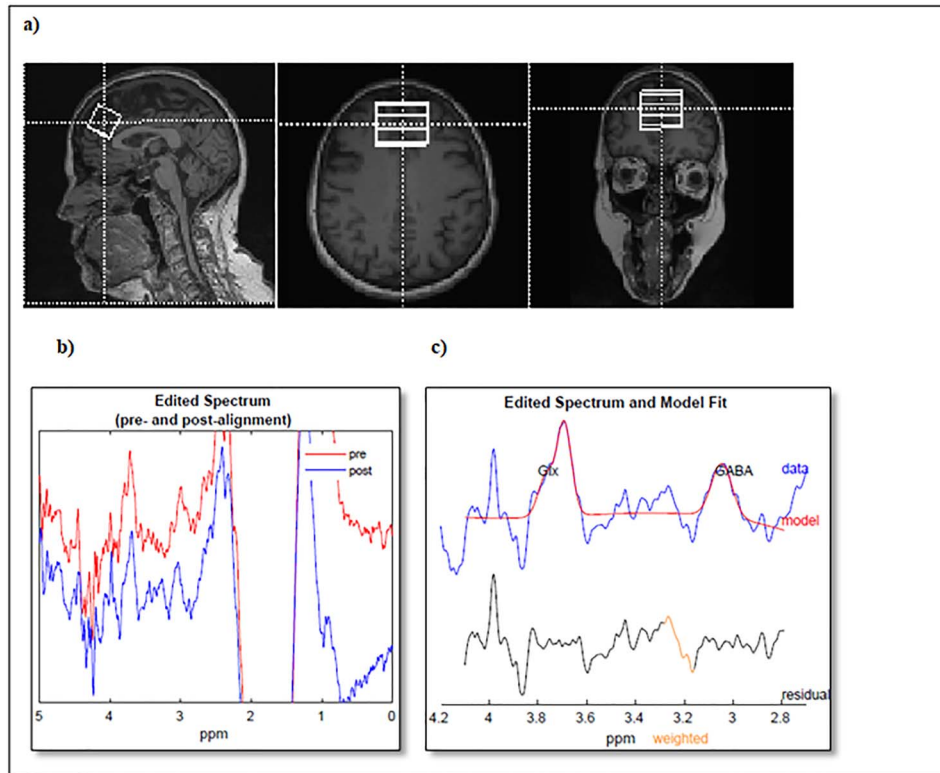
Figure 1. Data acquisition and processing. (*a*) Voxel placement of volume $25 \times 40 \times 25$ mm^3 in the dorsomedial/dorsoanterolateral prefrontal cortex ROI depicted by white solid boxes on the T_1-MRI sagittal, axial, and coronal planes. (*b*) Gannet Load output of processed GABA-edited spectra with preprocessed spectrum prior to frequency and phase correction (shown in red), and postprocessed spectrum after frequency and phase correction (shown in blue). (*c*) Gannet Fit output of fitted GABA signal with GABA-edited difference spectrum (shown in blue) with the overlapping model of best fit (shown in red) and residual between the spectrum and the model of fit (shown in black).

the localization of the voxel of dimensions $25 \times 40 \times 25$ mm^3 on our ROI in the axial, coronal, and sagittal planes through the center of the voxel in the superoinferior direction.

Images were acquired using a TIM Trio 3 T System (Siemens) employing a 12-channel head array coil transmission. MRS data were acquired using MEGA-PRESS sequence with the following parameters: TE = 68 ms, TR = 2600 ms, 32 averages, bandwidth = 1200 Hz, and total acquisition duration = ∼4 min. GABA-edited spectra were obtained by acquiring editing pulses at 1.9 ppm (edit pulse ON) and 7.46 ppm (edit pulse OFF). The estimation of in vivo GABA level in specific brain regions using noninvasive MRS technology is challenging due to the overlapping signals by higher concentrated metabolites such as choline (Cho), Cr, glutamate-glutamine (Glx), and N-acetyl aspartate (NAA) (Gao and Barker 2014). MEGA-PRESS aids in the removal of overlapping metabolites, and hence, we can detect free GABA signal co-edited with macromolecules (MM), that is, GABA+ signal at 3 ppm (Edden et al. 2014). The estimation of absolute GABA levels is challenging due to GABA's small quantity (1.3–1.9 mmol/kg) in brain. Notably, non–GABA-related factors, such as motion, can also influence GABA quantification. Thus, a normalization factor that is typically employed relative to Cr level or unsuppressed water signals at 3 ppm (Edden et al. 2014). We measured GABA levels relative to Cr signals as the GABA+/Cr levels have higher inter- and intra-individual reproducibility

compared with GABA+ relative to water signals (Bogner et al. 2010).

Data Processing and Analysis

Phase correction and spectral fitting of GABA+ signals were quantified by Matlab-R2018b-based Gannet v3.0 available at https://github.com/richardedden/Gannet3.0/archive/master.zip (Edden et al. 2014). This automated software gives output in 2 forms: GannetLoad and GannetFit. GannetLoad processes time-domain MRS data into the frequency-domain GABA edited spectrum in phase correction. GannetFit integrates the edited GABA+ signal at 3 ppm and provides the spectral fitting of GABA+ signal with reference to Cr. This fully automated software built on the MATLAB environment widens the accessibility and reduces the bias due to rater-dependent variances. Data with poor shimming quality due to subject motion or scanner drift were not processed by Gannet to yield the fitting output (Edden et al. 2014). GABA-edited spectra processed by Gannet were evaluated for their spectral quality based on the model of best fit overlying on the GABA+ spectrum as shown in Figure 1c.

T_1 volumetric segmentations and cortical reconstructions were processed using FreeSurfer v6.0 available at http://surfer. nmr.mgh.harvard.edu (Fischl and Dale 2000). We extracted whole brain gray matter (GM), white matter (WM), and ventricular

cerebrospinal fluid (CSF) volumes and included these as covariates in statistical analyses.

Neuropsychological and Behavioral Assessments

Participants were assessed using a comprehensive neuropsychological test battery which included the California Verbal Learning Test-II (CVLT-II) as a verbal memory measure (Delis et al. 2000). Because the neuropsychological battery was subsequently revised to minimize participants' fatigue, only a subset of the high-AD risk group ($n = 9$) completed the Brief Visuospatial Memory Test (BVMT) (Benedict 1997) and the Rey-Osterrieth Figure Drawing Test (Rey-O) (Goldstein and Naglieri 2011) as nonverbal memory measures.

Participants also completed the Geriatric Depression Scale (GDS) (Yesavage 1988), a validated depression scale for older adults, to characterize the sample and to be included as a covariate in statistical analyses.

Statistical Analyses

Statistical analyses were performed using SPSS v24.0. Due to nonnormality of the distribution of GABA+/Cr levels as determined by the Shapiro–Wilk test, we transformed GABA+/Cr levels using a two-step transformation method (Templeton 2011). In the first step, GABA+/Cr levels were transformed into percentile ranks to yield uniformly distributed probabilities. In the second step, inverse-normal transformation was applied to the percentile ranks.

To assess correlations between GABA+/Cr and memory measures, we used partial correlation controlling for age and whole brain tissue volumes of WM, GM, and CSF (Pizzi et al. 2017). Since alterations in the GABAergic system are well-established in depression (Sanacora et al. 1999), we repeated partial correlation analyses to include GDS scores as a covariate along with age and whole brain tissue volumes to assess the possibility that depressive symptoms accounted for any associations between GABA+/Cr level and memory.

Correlations were performed separately for the high-AD risk group and the CN group. For both groups, we correlated DM/DA-PFC GABA+/Cr levels with 3 verbal memory measures: CVLT-II Immediate Recall, CVLT-II Short-Delayed Free Recall, and CVLT-II Long-Delayed Free Recall. To reduce the probability of Type I error due to multiple comparisons, we applied a Bonferroni correction to yield a threshold of P value <0.017 for statistical significance ($0.05/3 = 0.017$).

Due to the limited number of participants who were administered with nonverbal memory tests, exploratory analyses were performed to examine the relationship between GABA+/Cr level and nonverbal memory measures (BVMT-Immediate and Delayed Recall, and Rey-O Immediate Recall) in the high-AD risk group ($n = 9$). As an even smaller number of CN participants completed the nonverbal memory tests, we were unable to perform correlations between GABA+/Cr levels and nonverbal memory measures in the CN group. For these exploratory analyses, we set the threshold at P value <0.05.

Results

Demographics, Clinical Characteristics, and Metabolite Compositions

Demographics and clinical characteristics of CN participants and the high-AD risk group, as well as SCD and MCI participants separately, are presented in Table 1. Relative to the CN group, depressive symptomatology as measured by GDS, was significantly greater in the high-AD risk group, as well as in the SCD group alone, although total GDS scores were still within the normal range (0–4) (Table 1) (Greenberg 2012). Mean GABA+/Cr level within DM/DA-PFC did not differ between the high-AD risk and CN or among SCD, MCI, and CN groups (Table 1).

Correlations between GABA+/Cr Level and Memory

High-AD Risk Group

Partial correlations between GABA+/Cr level and verbal memory controlling for age and GM, WM, and CSF volumes showed a robust correlation between GABA+/Cr and long-delayed free recall on the CVLT-II ($r = 0.70$, $P = 0.005$; Table 2a). Similarly, GABA+/Cr was significantly correlated with CVLT-II short-delayed free recall scores ($r = 0.64$, $P = 0.01$). A moderate correlation was observed between GABA+/Cr and immediate recall on the CVLT-II ($r = 0.60$, $P = 0.02$) but was not statistically significant at the Bonferroni-adjusted threshold of P value <0.017 (Table 2a).

Partial correlations between GABA+/Cr level and verbal memory controlling for GDS in addition to age, GM, WM, and CSF volumes showed that the correlation between GABA+/Cr and CVLT-II Long-Delayed Free Recall remained statistically significant ($r = 0.69$, $P = 0.009$; Table 2a, Fig. 2A). Although short-delayed and immediate recall scores on the CVLT-II were moderately correlated with GABA+/Cr level when GDS was covaried ($r = 0.62$, $P = 0.02$; $r = 0.57$, $P = 0.04$, respectively) as shown in Table 2a, these correlation coefficients were not statistically significant at the Bonferroni-adjusted threshold of P value <0.017.

Exploratory analyses in the subset of our sample who completed nonverbal memory tests showed a strong correlation between GABA+/Cr and immediate recall scores on the BVMT ($r = 0.99$, $P = 0.002$) after controlling for age, GM, WM, and CSF volumes (Table 2b). The strength of the correlation remained unchanged when GDS was included as a covariate ($r = 0.97$, $P = 0.03$; Table 2b, Fig. 2B). Strong correlations with r's >0.8 were similarly found between GABA+/Cr level and BVMT Delayed Recall and Rey-O Immediate Recall, in partial correlations analysis with age and GM, WM, and CSF volumes, with or without GDS, included as covariates. However, these findings were not statistically significant at the P value <0.05 threshold.

CN Group

No significant correlations were observed between GABA+/Cr levels and verbal memory in the CN group (Table 2a). As only 6 CN individuals completed the nonverbal measures, we were unable to perform partial correlations between GABA+/Cr and nonverbal memory.

Discussion

The purpose of our study was to assess the relationship between the GABA+/Cr level within DM/DA-PFC and memory in a sample of 24 high-AD risk and 14 CN participants. Although the limitation of sample size must be considered in interpreting the findings, our hypotheses were partially supported. Individuals in the high-AD risk group with higher GABA+/Cr level had greater long-delayed verbal recall, and this association remained statistically significant even after controlling for depression in addition to age and whole brain tissue volumes. Similar but less robust, associations were observed between GABA+/Cr level and

Table 1. Demographic details: Demographics and clinical characteristics, memory, and metabolite compositions in CN and the high-AD risk group, as well as SCD and MCI participants separately

Descriptive		Mean (standard deviation)		
	CN	High-AD risk group	SCD	MCI
	Demographics and clinical details			
Total sample size	14	24	11	13
Total number of females	9	11	6	5
Age in years	68.3 (6.4)	74.7 (6.9)**,a	75.1 (6.6)	74.3 (7.4)
Formal education in years	17.3 (3.2)	16.6 (2.4)	16.7 (2.6)	16.5 (2.3)
Montreal Cognitive Assessment scores	27.3 (1.5)**,c	25.7 (2.2)*,a	26.9 (1.9)*,c	24.7 (2.1)
GDS	0.6 (0.8)	1.8 (1.6)**,a	2.3 (2.0)**,a	1.5 (1.1)
	Neuropsychological measures			
California Verbal Learning Test-II (CVLT-II) Immediate recall scores	59.4 (8.8)***,b,c	40.8 (8.5)***,a	45.8 (8.9)*,c	36.5 (5.5)
CVLT-II short delayed free recall scores	12.4 (2.4)	8.2 (2.9)***,a	9.4 (2.8)*,a	7.2 (2.6)***,a
CVLT-II Long delayed free recall scores	12.6 (2.8)	8.3 (3.0)***,a	9.7 (2.8)*,a	7.1 (2.7)***,a
	Metabolite compositions			
GABA+/Cr levels	0.064 (0.04)	0.074 (0.05)	0.074 (0.04)	0.074 (0.06)

The details of SCD and MCI participants are present both individually and together as a group called high-AD risk. Data are presented as mean and standard deviations in brackets (). Single asterisk* represents P value <0.05, double asterisks** represent P value <0.01, and triple asterisks*** represents P value <0.001 for pairwise comparisons.
[a]Represents data significantly different from CN.
[b]Represents data significantly different from SCD.
[c]Represents data significantly different from MCI. One-way ANOVA with Bonferroni correction was applied to compare data between CN, SCD, and MCI participants. Independent t test with two-tailed equal variances was applied to compare data between CN and high-AD risk group which is a combination of SCD and MCI participants. CN and high-AD risk groups are shaded as they are the groups compared in statistical analyses.

Table 2a. Correlation analyses: Partial correlation between normally distributed GABA+/Cr levels within dorsomedial/dorsoanterolateral prefrontal cortex and verbal memory scores in CN ($N = 14$) and high-AD risk group ($N = 24$)

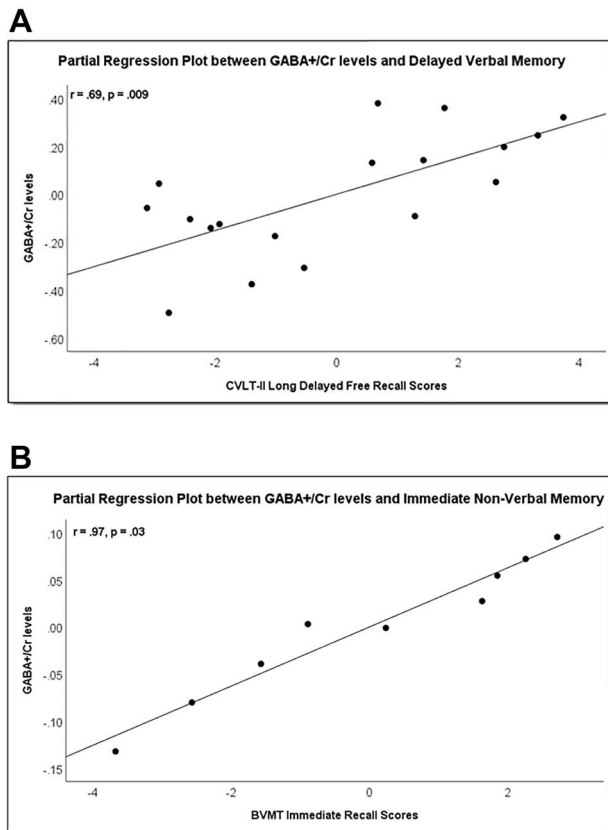| Scales	Subscales	Pearson's r (significance level)	
Covariates: Age and brain tissue volumes (WM, GM, and CSF)		CN	High-AD risk
California Verbal Learning Test–II (CVLT-II)	Immediate recall	0.26 (0.5)	0.60 (0.02*)
	Long delayed free recall	−0.24 (0.5)	**0.70 (0.005**)**
	Short delayed free recall	−0.25 (0.5)	**0.64 (0.01**)**
Covariates: Age, depression and brain tissue volumes (WM, GM, and CSF)		CN	High-AD risk
California Verbal Learning Test–II (CVLT-II)	Immediate recall	0.40 (0.3)	0.57 (0.04*)
	Long delayed free recall	−0.18 (0.7)	**0.69 (0.009**)**
	Short delayed free recall	−0.21 (0.6)	0.62 (0.02*)

The correlation analyses are repeated with depression scores as a covariate in addition to age and whole brain tissue volumes. The Bonferroni-adjusted significance level P value <0.017 is applied here. Bonferroni-adjusted significance levels (P value < 0.017) are represented with double asterisks** and bold format. Significance levels (P value < 0.05) are represented with single asterisk*.

Table 2b. Correlation analyses: Partial correlation between normally distributed GABA+/Cr levels within dorsomedial/dorsoanterolateral prefrontal cortex and nonverbal memory scores in the subset of high-AD risk participants who completed the nonverbal memory measures ($N = 9$)

Scale	Subscale	Pearson's r (significance level)
Covariates: Age and brain tissue volumes (WM, GM, and CSF)		High-AD risk
BVMT	Immediate recall	**0.99 (0.002**)**
	Delayed recall	0.83 (0.08)
Rey-Osterrieth	Immediate recall	0.86 (0.06)
Covariates: Age, depression, and brain tissue volumes (WM, GM, and CSF)		High-AD risk
BVMT	Immediate recall	**0.97 (0.03*)**
	Delayed recall	0.79 (0.21)
Rey-Osterrieth	Immediate recall	0.87 (0.13)

The correlation analyses are repeated with depression scores as a covariate in addition to age and whole brain tissue volumes. The number of CN participants who completed these tasks was too small to perform similar partial correlations. Significance level P value <0.05 is applied to these exploratory analyses. Significance levels (P value < 0.01) are represented with double asterisks** and bold format. Significance levels (P value < 0.05) are represented with single asterisk* and bold format.

A

Partial Regression Plot between GABA+/Cr levels and Delayed Verbal Memory

r = .69, p = .009

B

Partial Regression Plot between GABA+/Cr levels and Immediate Non-Verbal Memory

r = .97, p = .03

Figure 2. (A) Partial regression plot between GABA+/Cr levels and verbal memory. Higher GABA+/Cr levels were associated with better verbal memory performance as measured by CVLT-II Long-Delayed Free Recall in the total sample of high-AD risk participants ($n = 24$). GABA+/Cr levels are plotted against CVLT-II Long-Delayed Free Recall scores with age, depression, and brain tissue volumes (GM, WM, and CSF) as covariates. The straight line depicts the line of fit demonstrating the strength of this correlation ($r = 0.69$, $P = 0.009$). (B) Partial regression plot between GABA+/Cr levels and nonverbal memory. Higher GABA+/Cr levels were also associated with better nonverbal memory performance as measured by BVMT Immediate Recall in the subset of high-AD risk participants ($n = 9$) who completed these tests. GABA+/Cr levels are plotted against BVMT Immediate Recall scores with age, depression, and brain tissue volumes (GM, WM, and CSF) as covariates. The straight line depicts the line of fit demonstrating the strength of this correlation ($r = 0.97$, $P = 0.03$).

CVLT-II immediate recall and short-delayed verbal recall scores. We also found evidence of strong positive associations between GABA+/Cr level and nonverbal memory, with a statistically significant correlation found for immediate recall on the BVMT-only. These associations appear to be specific to older adults at high-AD risk since similar findings were not observed in the CN group, in which no significant correlations were detected between GABA+/Cr level and memory measures. To the best of our knowledge, the current study is the first to report a strong association between DM/DA-PFC GABA+/Cr level and memory in older adults at high-AD risk.

Previous studies in CN older adults have linked enhanced excitatory glutamate/glutamine (Glx) levels in hippocampus with better verbal memory processes (Nikolova et al. 2017) and reduced inhibitory GABA levels in frontal cortex with cognitive decline (Porges, Woods, Edden, et al. 2017a). Our findings of an association between GABA+/Cr within DM/DA-PFC and memory in the high-AD risk group are consistent with these earlier studies. The present findings also extend the extant literature

regarding reduced GABA in AD by demonstrating that the reduction in GABA appears to be tightly connected to verbal and nonverbal memory. This is in line with the established link between GABAergic and cholinergic systems. The GABAergic system stimulates the cholinergic neuronal system which is disrupted by AD pathology (Cummings and Back 1998), and it has been suggested that alterations in GABA level may represent a compensatory response to disruption of cholinergic networks in AD.

A major strength of our study is that participants were well-characterized with respect to cognition and mood, and none had a previous or current psychiatric disorder. This is an important methodological detail because alterations in GABA levels are found in depression, anxiety, and other psychiatric disorders (Kalueff and Nutt 2007; Luscher et al. 2011). Thus, the findings in our sample of high-AD risk participants are not likely to be confounded by underlying current or remitted psychiatric illness. A second strength of our study is that our statistical approach considered the impact of the whole brain tissue volumes, including GM, WM, and CSF on the GABA+/Cr level to reduce potential inter- and intra-individual variations of tissue components based on brain segmentation (Porges, Woods, Lamb, et al. 2017b). This is important because metabolite levels are known to vary across different brain tissues with higher distribution of GABA in GM compared with WM (Porges, Woods, Lamb, et al. 2017b).

Our study is not without limitations. As highlighted earlier, the main limitation is the small sample size, a consequence of the number of scans excluded from analysis due to movement artifacts, and hence poor spectral quality in both the high AD-risk (11 out of 35 scans) and CN groups (13 out of 27 scans). Thus, the extent to which our findings are stable and generalizable is unclear. The small sample sizes may have also precluded detection of statistically significant correlations in the CN group, and for some of the findings in the AD-risk group, where moderate-to-high correlation coefficients were observed (r's in the range of 0.3–0.7) but not statistically significant. Due to the small numbers of SCD and MCI participants, both were combined into a single high-AD risk group for the purposes of data analyses. Thus, we are unable to establish the specificity of our findings to SCD or MCI. Finally, there are methodological limitations with the scanning parameters we used to acquire MRS images. Our scans included 32 averages, which is the minimum number of averages recommended by guidelines published after we had begun acquiring MRS data (Mikkelsen et al. 2018) but clearly does not meet current 2020 guidelines which specify a minimum of 240 averages (Peek et al. 2020). In addition, our acquisition time of ~4 min falls short of the recommended 7–10 min (Mullins et al. 2014). This shorter acquisition time was selected to optimize both power and the feasibility of older adults remaining as still as possible for the duration of the scan. This was a challenge for older adults with lower back pain or other pain syndromes and is reflected in the number of unusable images due to movement artifact.

Nevertheless, we report novel findings that show a direct relationship between GABA+/Cr level in the DM/DA-PFC and delayed verbal memory in high-AD risk individuals. Given that delayed verbal memory is a robust predictor of cognitive decline progressing from MCI to AD (Rabin et al. 2009), it is noteworthy that the strongest association observed was between GABA+/Cr level and CVLT-II long-delayed recall scores. These findings extend the literature reporting reduced GABA overall in AD by demonstrating that GABA level is closely linked to cognitive biomarkers of AD. Although the current results need to be replicated using larger sample sizes, our findings provide support for further

investigations of the GABAergic system to discover novel biomarkers and interventions for AD.

Notes

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