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3T Hippocampal Glutamate-Glutamine Complex Reflects Verbal Memory Decline in Aging

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Abstract

The hippocampus is a critical site for alterations that are responsible for age-related changes in memory. Here, we present a relatively novel approach of examining the relationship between memory performance and Glutamate-Glutamine levels using short echo time magnetic resonance spectroscopy (MRS). Specifically, we investigated the relationship between Glx (a composite of glutamate and glutamine) levels in the hippocampus, performance on a word recall task and resting state functional connectivity (RSFC). While there was no overall difference in Glx intensity between young and aging adults, we identified a positive correlation between delayed word-list recall and Glx, bilaterally in older adults, but not in young adults. Collapsed across age, we also discovered a negative relationship between Glx intensity and RSFC between the anterior hippocampus and regions in the subcallosal gyrus, replicating a recent finding by Wagner et al., (2016). These findings demonstrate the possibly utility of Glx in identifying age-related changes in the brain and behavior and provide encouragement that MRS can be useful in predicting age-related decline before any physical abnormalities are present.

Keywords

3T MRI; aging; MRS; memory; imaging; Glx

1. Introduction

One of the key concerns of older adults is the experience of memory loss, both in the normal course of aging and as it is associated with neural degenerative disease. Many of the types of memory that demonstrate age-related decline are mediated by the hippocampus and surrounding medial temporal lobe structures (Morrison and Baxter, 2012; Small 2001; Yassa et al., 2011a). While there are many laboratory tasks that are designed to tax specific aspects

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of hippocampal function, standardized neuropsychological tests are broadly available and extensively used in both research and clinical settings. The Rey Auditory Verbal Memory Task (RAVLT; Rey, 1941) has been shown to be highly effective at detecting age-related memory decline (Mitrushina et al., 1991; Schoenberg et al., 2006). In this task, memory is assessed based on the acquisition of a list of 15 unrelated words, followed by free-recall, and repeated over the course of 5 presentations. Participants are then given an interference list of 15 new words, followed by free recall of the original list and another free recall 15 minutes later. Age-related decline has been observed on all recall trials (Mitrushina et al., 1991), with performance on delayed recall correlating with functional scales of memory in those with subjective memory complaints (Estevez-Gonzalez et al., 2003). It is currently used by a number of research groups to identify healthy agers who are particularly “successful” (Mapstone et al., 2014), “super” (Rogalski et al., 2013), or “unimpaired” (Stark et al., 2013) in their memory performance relative to other, healthy participants. Likewise, a delayed word-recall performance on a related task is particularly sensitive to early decline associated with Alzheimer’s disease (Albert 1996).

While the aging brain has been associated with declines in hippocampal volume (Raz et al., 2015), there is strong evidence that this neuronal loss is not simply due to fewer neurons (Burke & Barnes, 2006). Instead, there is evidence of synaptic alterations (Nicholson et al., 2004) and decreases in gene expression (Berchtold et al., 2013) in aging. Likewise, there are neuromodulatory changes in the hippocampus, including decreased cholinergic input to the hippocampus (Sugaya et al., 1998) and a reduction in dopamine that correlates with memory performance (Stemmelin et al., 2000). While these findings have been reporting in rodents, we now have the technology to investigate some of these neurotransmitters in humans using magnetic resonance spectroscopy (MRS). Instead of looking at MRS related to disease, the goal of this work is to link it to behavioral performance.

MRS quantifies the concentration of various metabolites (intermediary and byproducts of metabolism) in the brain, with many of these accessible via conventional proton (^1H) imaging. Numerous studies have utilized the efficiency of MRS in providing biological information on cellular/metabolic changes in the aging brain or diseased brain (see Wang et al., 2015 for a review). Specifically, our focus is on Glutamate (Glu), the most abundant metabolite in the brain, a dominant excitatory neurotransmitter. Glutamate is released during neuronal excitation and converted to Glutamine (Gln) with the Glutamate-Glutamine cycle requiring high energy demands. Alterations in concentrations of Glu and Gln have been reported in numerous neurological and psychiatric diseases, such as depression and mood disorders, epilepsy, alcohol and drug abuse, schizophrenia, and neurodegenerative disorders (see Ramadan et al., 2013 for a review).

MRS studies have shown reduced Glu levels in a mouse model of Alzheimer’s disease (AD) (Chen et al., 2012b) and in humans with AD (Rupsingh et al., 2011). They have also shown a decrease in combined Glu and Gln (referred to as Glx) in the cingulate cortices and posterior cingulate gyrus of AD patients (Hattori et al. 2002; Antuono et al. 2001). Little is known about any such alterations in healthy aging or in the hippocampus *per se*, despite the clear potential for age-related changes in hippocampal activity (Miller et al, 2008; Wilson et al., 2006; Yassa et al., 2011b). In one report, Wagner et al. (2016) investigated the

relationship between hippocampal Glx, functional connectivity with the hippocampus, and verbal memory performance (on a different task) in healthy young males. They reported that lower Glx levels were associated with higher functional connectivity of the hippocampus to prefrontal cortex and anterior cingulate. Additionally, they found that Glx concentration in the posterior hippocampus predicted verbal memory performance. These relationships emphasize the utility of MRS as a functional measure of neuronal activity, related to both behavior and brain connectivity.

Given the relationship of Glx to aging and AD, and the possible link to memory performance, the purpose of this study was to investigate age-related changes in Glx levels in the hippocampus using short echo time MRS in young and older adults. We hypothesized that Glx levels would correlate with age-related decline on the RAVLT. Following the findings of Wagner et al. (2016), we also investigated the relationship between Glx and resting state functional connectivity (rs-fMRI) in a subset of our sample for which we were fortunate enough to also have rs-fMRI scans. We hypothesized that we would replicate the negative relationship found between the RSFC anterior hippocampus to regions in the prefrontal cortex and Glx levels in the right anterior hippocampus. These findings would significantly extend those of Wagner et al. (2016) by showing the behavioral relevance of Glx in the hippocampus to healthy aging and link them to the highly-used RAVLT.

2. Materials and Methods

2.1 Participant Recruitment

Forty-one participants, consisting of 21 older (ages 59–84) and 20 younger adults (ages 20–38) were recruited for this study from the community surrounding the University of California in Irvine (UCI). Two participants were removed from the analysis: one young adult was excluded because one of their MRS voxels contained only 20% overlap with the hippocampus (motion between scan preparation and acquisition) and one older adult was excluded for RAVLT scores that fell outside the age-thresholded norms. Thus, we analyzed data from 20 older (8 males, 12 females, mean age of 70 ± 6 years) and 19 younger (8 males, 11 females, mean age of 27 ± 5 years) adults. All participants were right-handed, had no history of neurological disease or psychiatric illness, and had normal or corrected-to-normal vision. Additionally, they all performed within the normal range for their age on standardized neuropsychological testing and scored in the normal to mild range on the Geriatric Depression Scale. All participants gave informed consent approved by the UCI Internal Review Board prior to participation, and were compensated for their time.

Verbal memory was evaluated using the Rey Auditory Verbal Learning Test (RAVLT; Rey, 1941). The RAVLT involved learning a list of 15 unrelated words, repeated 5 times, with a recall test after each administration of the list. Following the 5 presentations, a new interferences list of 15 words was administered, along with a recall task. After the interference list, participants were then asked to recall the original list for an immediate recall test and assessed again 15-minutes later with a delayed recall test.

2.2 MRS Data Acquisition

MRS scans were collected using a 3.0T Philips whole body MRI (Best, the Netherlands), using a 32 channel head coil. High resolution T1-weighted and T2-weighted images were acquired parallel to the hippocampus.

T₁-weighted whole-brain anatomical images were acquired using a sagittal magnetization-prepared rapid gradient echo scan (MP-RAGE) at a resolution of 0.75 mm (isotropic), repetition time (TR)/echo time (TE) = 11/4.6 ms, field of view (FOV) = 320 × 264, and flip angle = 18°. In addition, a T2-weighted whole-brain anatomical (TR/TE = 8/4.6 s) scan was used as an anatomical reference to localize the hippocampal voxel placement for each participant. The rectangular voxel of interest was placed along the long axis of the hippocampus on the sagittal T₂ image and contained mainly the anterior hippocampus (Figure 1).

¹H spectroscopy data were acquired using PRESS (TR/TE = 2000/37 ms), from a nominal 2.5 × 1.5 × 1.0 cm³ voxel in the left and right hippocampi of all subjects. The acquisition of MRS spectra took about 8 minutes out of a total MR time of 60 min per subject. Voxel dimensions were chosen to maximize the volume while largely contained within the anterior hippocampus of each subject. Hippocampal volumes were calculated using Freesurfer (see MRS Data Analysis section). For the right hippocampus the values ranged between 3.0 cm³ to 3.75 cm³ (mean and SD 3.3 ± 0.6 cm³ in aged subjects and 3.70 ± 0.2 cm³ for young participants). For the left hippocampi the volumes varied between 2.4 cm³ to 4.06 cm³ (mean and SD 3.3 ± 0.6 cm³ in aged subjects and 3.1 ± 0.6 cm³ for young participants). Shimming was accomplished using the iterative VOI method provided by Philips. Only shims below 10 Hz were included in the study. MRS data for five participants were excluded because either of the linewidths (left or right hippocampus) exceeded 10 Hz.

2.3 rs-fMRI Data Acquisition

We were fortunate to have resting-state data from 22 participants (13 young and 9 older adults), collected on a separate day. Echo-planar images were collected on a separate day from the MRS scans using the same scanning equipment. Whole-brain resting state data were acquired with a T₂* weighted echo-planar imaging scan, at a resolution of 2.5mm (isotropic), 46 axial slices, TE = 26ms, flip angle = 70°, TR = 2500ms, dynamics = 188. The resting-state scan took 7 minutes 27 seconds, during which time participants were instructed to relax with their eyes open. Using AFNI (<http://afni.nimh.nih.gov/afni>; Analysis of Functional NeuroImages). The data were aligned to the participant's MP-RAGE with the script `align_epi_anat.py` (Saad et al., 2009). Each participant's structural scan and functional data (statistical maps) were aligned to a model template using ANTS (Advanced Normalization Tools; Avants et al., 2008).

2.4 MRS Data Analysis

Quantitative MRS data was initially analyzed with TARQUIN v.4.3.6 (Wilson et al. 2011), using a non-negative least squares projection to estimate signal amplitudes. Initial point truncation and HSVD water removal was used to reduce baseline interferences (Wilson et al. 2011).

The fractional contributions of gray matter (GM), white matter (WM), and cerebrospinal fluid (CSF) were determined for each voxel by segmenting the T_1 weighted images using SPM8 (SPM-Statistical Parametric Mapping 2016; Ashburner and Friston 1997) and MATLAB (the Mathworks, INC. Natick, MA). The fractional tissue contributions were used to correct the estimated metabolite concentrations for partial volume effects (Gussew et al. 2012) using a customized MATLAB code. The partial volume correction required relaxation rates (T_1 and T_2) of water and metabolites in both WM and GM (Stanisz et al. 2005).

Freesurfer was used to segment the T_1 -weighted images into hippocampal volumes. The percent of each voxel that overlapped with the hippocampus was calculated and reported in Table 1. The percent of MRS voxel GM consisting of hippocampal tissue was recorded as well. The mean MNI coordinates of each voxel were determined first by warping each anatomical T_1 to MNI152 template using Advanced Normalization Tools (ANTs; Avants et al., 2008), and subsequently by applying the warp transform to each voxels 3D binary mask (1mm isotropic resolution). The mean MNI coordinates were calculated from the warped binary mask in MNI space. Hippocampal volumes and ventricular volumes were also calculated on the MP-RAGE for each participant using Freesurfer's automatic software for volumetric measures (FreeSurfer 2016; Fischl et al. 2002). These volumes were normalized by dividing by total intracranial volume for each participant.

2.5 rs-fMRI Data Analysis

Data were preprocessed using AFNI and included detrended using 2nd order Legendre polynomials. The time series was then normalized to have zero mean and unit variance and the motion vectors and first derivatives were regressed out of the signal. The first 6 principal components of the variance were computed using CompCor (not including global signal) and regressed out of the signal (Behzadi et al., 2007). The data were then temporally bandpass filtered (0.009 to 0.08 Hz) and TRs with framewise displacement >0.5mm, as well as those one TR before and two TRs after, were removed from analyses by censoring as recommended by Power et al., (2012). Thus, the remaining data for both the young and older adults contained similar, very low levels of motion. Note, all rs-fMRI analyses to identify regions showing reliable connectivity to the hippocampus were done on the entire set of participants, regardless of age.

We estimated the inherent blur using 3dFWHMx (AFNI) for each participant and used the mean of these values in 3dClustSim¹ (AFNI: June 2016) to determine the combined voxel-wise ($p < 0.005$) and cluster-size (nearest-neighbor connectivity of 10 contiguous voxels) thresholds needed to arrive at a multiple-comparison corrected alpha of $p < .05$.

2.6 RAVLT Data Analysis

Free recall from the 5 acquisition tests in the RAVLT were summed to produce a RAVLT Total Score. In addition, the RAVLT Interference score was calculated (total recalled from the Interference word list administered after these). Finally, we also scored the total recalled

¹3dClustSim was updated in May 2015 to address an error in underestimating multiple corrections, resulting in an overestimation of significance for a given cluster (Eklund et al., 2016). Here, we have used the updated version of 3dClustSim, which corrected this error.

from the Immediate Recall and the Delayed Recall, administered 15 minutes later. We used Prism 6 (www.graphpad.com) to calculate unpaired t-tests for between group comparisons and correlations between behavior and Glx values.

3. Results

3.1 Age-related differences in RAVLT performance

One older adult performed more than 2 standard deviations below standardized normative values for his age. As this may represent either exceptionally inattentive performance or potential early clinical impairment, this individual was excluded from further analysis. All other young and older participants performed within 1 standard deviation of the standardized norms for their age.

In the resulting dataset, we examined the effect of aging on the RAVLT using planned independent samples t-tests. As expected, we found that older adults performed worse than young adults on all four measures of the RAVLT: Total Acquisition, Interference, Immediate and Delayed Recall (see Table 1).

3.2 No age-related differences in Glx concentration

Tissue fractions and normalized hippocampal and ventricular volumes for both groups are shown in Table 1. There was no evidence for differences in the proportion of GM, WM, and CSF in our MRS voxels across age groups, reducing potential concerns for artefactual differences in metabolite concentrations resulting from varying partial volume effects across groups. Consistent with a wide range of studies, both left and right hippocampal volume, as well as ventricular volume, were lower in the aging group. Not surprisingly, the average volume of the MRS voxels were also smaller in aged than young participants. However, the average Glx, presented in absolute concentrations (mmol/L), did not differ between the two groups. Thus, while hippocampal atrophy was observed in the aging group, we were still able to obtain a robust measure of Glx.

3.3 Age-related differences in MRS and RAVLT performance

To reduce noise and as we had no *a priori* hypotheses concerning laterality, we began investigating the relationship between Glx and memory performance by averaging the left and right Glx values for each participant, correlating the result with RAVLT performance. We found significant positive correlations between Glx and RAVLT Total, Immediate and Delayed Recall for the aged group (Figure 2). There was no evidence for such relationship in the young group.

Since MRS was collected from a single, often unilateral voxel, our combination of the two hemispheres' Glx values in our initial analysis is somewhat atypical. Thus, as a secondary analysis, we analyzed these relationships separately for each hemisphere to determine if the results were consistent and replicable in each. Similar, independent correlations were observed in each hemisphere in the aging group between Glx and RAVLT Total (Left: $r = 0.62$, $p < 0.01$; Right: $r = 0.39$, $p = 0.09$), Immediate (Left: $r = 0.54$, $p < 0.05$; Right: $r = 0.52$, $p < 0.05$) and Delayed Recall (Left: $r = 0.65$, $p < 0.01$; Right: $r = 0.42$, $p = 0.07$). No

relationship between Glx and RAVLT performance was observed in the young group (all p 's > 0.5). We note that this lack of a relationship in our anterior hippocampal voxels is consistent with the findings of Wagner et al. (2016). While they observed a relationship between hippocampal Glx and performance on a test that provides a similar measure of memory as the RAVLT Interference score, this relationship was only found for posterior and not anterior hippocampal voxels.

3.4 Relationship between RSFC and Glx

Resting State Functional Connectivity (RSFC) analyses were carried out by correlating the regional signal-time courses extracted from the left and right anterior hippocampus (corresponding to the regions where the MRS scans were collected), to all other voxels in the brain. We applied Fisher z-transformation to the linear correlation maps prior to performing statistical analyses. Due to the reduced number of participants in the young and aging groups who had both MRS and rs-fMRI, we collapsed the young and older adults into a single group.

Using 3dRegAna (AFNI), we tested the relationship between hippocampal RSFC (separately for the left and the right anterior hippocampus) and Glx intensity. There were 14 regions that revealed a relationship between left or right RSFC and Glx concentration presented in Table 2 (multiple-comparison-corrected). Of these, two of the regions (Figure 3) were located in the left subcallosal gyrus, near the orbitofrontal cortex (OFC) location identified by Wagner et al. (2016). In both of these regions, we replicated the Wagner et al. (2016) finding of a negative relationship between Glx concentration and RSFC from the anterior hippocampus to the OFC. Interestingly, while the Wagner group found this relationship in young adult males, we extended these findings in a group of healthy, mixed gender, older and younger adults. We (post hoc) plotted the young and older adults separately in Figure 3 to demonstrate the consistency in both groups but, given our small sample size, we did not contrast them directly. In addition to the two OFC regions that overlapped with those reported by Wagner et al. (2016), we also identified 12 other regions, displaying a mix of positive and negative relationships between Glx intensity and RSFC.

4. Discussion

In this study, we measured Glx (a combined measure of glutamate and glutamine) in the anterior hippocampus of young and older adults. We found that greater Glx concentration in older adults was correlated with higher memory performance in a word recall on the RAVLT, both at encoding and retrieval. Moreover, we showed that this effect was observed in both the left and the right hippocampus, providing an internal replication of this relationship. Interestingly, this relationship was not evident in young subjects.

These findings extend those of Wagner et al. (2016), who reported a positive correlation in young males between hippocampal Glx levels and retroactive interference on a task similar to the RAVLT. Our results are largely consistent and complementary here, as the studies contain both overlapping and independent measures. Briefly, Wagner et al. (2016) observed their Glx — verbal memory correlation in posterior but not anterior Glx measures and only in the Interference memory measure. In addition, they only scanned young males. Our data

from the anterior hippocampus failed to show a relationship between Glx and verbal memory consistent with their observations. As we do not have MRS data from the posterior hippocampus, we cannot address whether young individuals show a similar relationship with the Interference score of the RAVLT. While it is by no means clear, there is growing evidence for some form of anterior-posterior distinction in the hippocampus (Strange et al., 2014). For example, activity in the posterior hippocampus has been shown to predict the degree of retroactive interference in a paired-associates task (Kuhl et al., 2010), highlighting the relevance of this region for retroactive interference specifically. We collected data from the anterior hippocampus, which has been associated with novelty detection (Daselaar et al., 2006) and a relationship to the dorsal attention network (Kim, 2015). Thus, the anterior hippocampus may be more sensitive to the recall measures of the RAVLT than to retroactive interference. We caution, however, that such interpretations are entirely speculative and based on reverse inferences at this stage.

We did, however, replicate their basic effect by demonstrating a relationship between hippocampal Glx and RAVLT performance. In our study, we found this relationship in the anterior hippocampus in a heterogeneous group of older adults for several of the verbal learning measures. Note, Wagner et al. (2016) did not test older individuals or women. Thus, we view our results as being consistent with and complementary to those of Wagner et al. (2016) in this regard.

Our observation of a relationship between Glx levels and RAVLT performance in aging adults suggests a possible modulatory effect of glutamate and/or glutamine on memory performance. Higher levels of glutamate in the striatum have been associated with better performance on tests of executive functioning in a group of older adults, provided a potential mechanism for age-related decline in the frontostriatal system (Zahr et al., 2008). N-methyl-D-aspartate (NMDA) receptors represent one of the glutamate receptors that are present in high density in the hippocampus and cerebral cortex, even in aging and Alzheimer's disease (Cotman et al., 1989), but there is mounting evidence that NMDA receptor function declines with age (Barnes et al., 1997). Likewise, an age-related deficiency in NMDA receptor function contributes to impairments in spatial learning and memory in older rodents (Magnusson 1998; Zhao et al., 2009), providing a mechanism for glutamate-related dysfunction in aging that may be contributing to a decline in verbal memory.

As noted earlier, in our current data we are unable to separate the Glu and Gln components of the signal and, like many studies, must combine this into a Glx value. Glutamate and glutamine are complementary, such that glutamate is taken up in the synaptic cleft and then converted to glutamine within the astrocytes. This cycle is rapid and highly dynamic, with Glu located primarily in neurons and Gln located predominately in the glial cells (Ottersen et al., 1992). While we found no age-related decrease in Glx concentration between young and older adults in the hippocampus, other studies have found evidence for an age-related decline in Glu, accompanied by an age-related increase in Gln in the hippocampus (Hadel et al., 2013; Schubert et al., 2004). These opposing effects may have mitigated any age-related difference in overall Glx concentration here, but an underlying age-related imbalance may still account for the relationship with RAVLT performance. Outside of the hippocampus, age-related decreases in glutamate have been reported in the striatum (Zahr et al., 2008; but

see Choi et al. (2014) for the opposite pattern), but not the cerebellum (Zahr et al., 2008, 2013), emphasizing the possible regional specificity of these metabolic imbalances. Additionally, the opposing dynamics of the glutamate-glutamine cycle may be completely masked by the combined Glx metric. Future studies using acquisition techniques that can isolate Glu and Gln (e.g., Mega-PRESS sequences) are needed to identify the opposing contributions of these two metabolites.

The relationship between Glu and Gln concentrations in aging is a complicated one. These compounds reflect both neuronal and mitochondrial energy production, with Glu serving as a metabolic precursor of γ -aminobutyric acid (GABA), the main inhibitory neurotransmitter in the cerebral cortex. Mitochondrial dysfunction in normal aging may lead to an increase in Glu (Boumezbeur et al., 2009; Lin & Beal, 2006) and be a major contributor to the underlying cause of AD (Lin et al., 2003). Further, ameliorating this mitochondrial dysfunction has been shown to improve cognitive performance in rodent models of AD (Chen et al., 2012a). Increased hippocampal Glu levels have also been observed in a number of disorders, including medial temporal lobe epilepsy (Simister et al., 2002, Woermann et al., 1999) and schizophrenia (Kraguljac et al., 2014). Based on these findings, we might have predicted that greater Glu levels would be associated with worse memory performance on the RAVLT in older adults. In contrast, we find that for healthy older adults, greater Glx concentration is associated with greater memory performance. One might speculate that higher Glx values reflect greater regulation of the Glu/Gln cycle, since the combined Glx measure does not allow us to analyze the individual contributions of Glu and Gln to memory performance. Yet, any speculation here must be tempered by the fact that overall Glx concentration did not differ between the older and young adults, therefore the relationship between Glx and memory performance in the older adults may not reflect a global shift in Glu associated with clinical conditions such as schizophrenia, epilepsy, and AD. Notably, in these diseased states, the decrease of Glu is consistently accompanied by a decrease in NAA (Bartha et al., Rupising et al. 2009 and Jessen et al. 2001), signifying neuronal loss or dysfunction. In this study, a decrease in NAA was not observed or expected as healthy aging is not associated with overt neuronal loss in the hippocampus (Burke & Barnes, 2006).

In this study, we also replicated another finding reported by Wagner et al. (2016) of a negative relationship between hippocampal Glx in the anterior hippocampus and the RSFC from the anterior hippocampus to regions in the orbitofrontal cortex (OFC). The OFC is one of the regions of the default mode network (DMN), a network of highly interconnected brain structures that show synchronous low-frequency fluctuations during a resting state condition (Raichle et al., 2001). Previous correlations between Glx and nodes of the DMN have been observed, particularly with increased glutamate levels in the anterior cingulate cortex (Enzi et al., 2012) and the posterior cingulate cortex (Duncan et al., 2013; Hu et al., 2013). Thus, the inter-regional synchrony in the DMN may be modulated, at least in part, by the excitation-inhibition balances based on the ratio of Glu and Gln.

In contrast to the findings of Wagner et al. (2016), it is worth noting that we also found both negative and positive relationships between Glx and RSFC between the anterior hippocampus and other regions outside of the DMN, including regions in the

parahippocampal gyrus, parietal cortex, and cerebellum. Only a few studies have investigated the relationship between glutamate levels and task-induced BOLD signal or RSFC in humans. Using MRS in the dorsal anterior cingulate cortex, Falkenberg et al. (2012) categorized young healthy adults into high and low glutamate individuals and showed that those with low glutamate levels showed an increased BOLD response during a task that demanded low cognitive control, whereas individuals with high glutamate levels showed the opposite effect. Similarly, a positive relationship between RSFC and glutamate levels in the anterior cingulate cortex have been shown in young adults. These findings emphasize an interaction between glutamate levels and the activation levels of these regions, though they have been limited to very specific regions of interest.

Glutamate, as an excitatory neurotransmitter, may result in high levels of RSFC in regions that are task-activated, while a reduction in this glutamatergic activity, or a counteraction of it by an inhibitory neurotransmitter such as GABA, may lead to task-induced deactivation. These relationships are highly dependent on the task and the regions involved in the task, but reflect the utility of these metabolites as functional measures of neuronal activity. In this study, the rs-fMRI data was collected during “rest”, which is a cognitively uncontrolled condition that results in high BOLD activity in the hippocampus (Stark & Squire, 2001). The complex relationship between how glutamate levels in the hippocampus modulates rest-based RSFC is beyond the scope of this investigation. Instead, we report these relationships to 1) report on a replication with Wagner et al. (2016), 2) demonstrate a relationship between Glx and RSFC across many regions, and 3) reveal the complexity of this relationship across regions. With a larger sample size or multiple MRS voxels, it would be interesting for future studies to investigate any age-related modulation of these relationships.

This study was limited by a small sample size for the rs-fMRI dataset, which prevented us from examining any age-related differences in the relationship between Glx and resting state activity. We were also limited in our ability to contrast Glx and network relationships because we collected MRS from only the anterior hippocampus. In future studies, collecting MRS voxels from the anterior and posterior hippocampus, or from other memory-related regions such as the parahippocampal gyrus, would provide an opportunity to compare the relationship between Glx and functional activity. Finally, since Glx is a composite measure, we are unable to distinguish the individual contributions of glutamate and glutamine to the behavior or the brain activity. The use of a MEGA-PRESS sequence may be able to differentiate these two metabolites in the future. GABAergic inhibition plays a key role in glutamatergic excitation (McCormic et al. 1989), and the balance of is essential to consider with respect to hippocampal function and memory. In MRS, the Glutamate and GABA peaks however overlap, making it difficult to separate those metabolites without the use of J-editing spectral techniques. We were unable to investigate the effects of GABA here since special sequences are needed to resolve this peak but would like to address the role of GABA in future work.

5. Conclusions

We report that decreases in Glx in the anterior hippocampus were accompanied by decreased performance in verbal memory in aged adults. These preliminary findings suggest a

potential age-related modulatory role of glutamate and/or glutamine in the hippocampal regulation of memory. Future studies are needed to focus on how a dysregulation of the excitation-inhibition balance in the hippocampus may contribute to age-related memory decline.

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Highlights

- Correlation between Glx and verbal memory performance in older adults, but not younger adults
- Glx in the anterior hippocampus is related to resting state fMRI in several regions
- Glx may provide an age-related modulatory role in the hippocampal regulation of memory

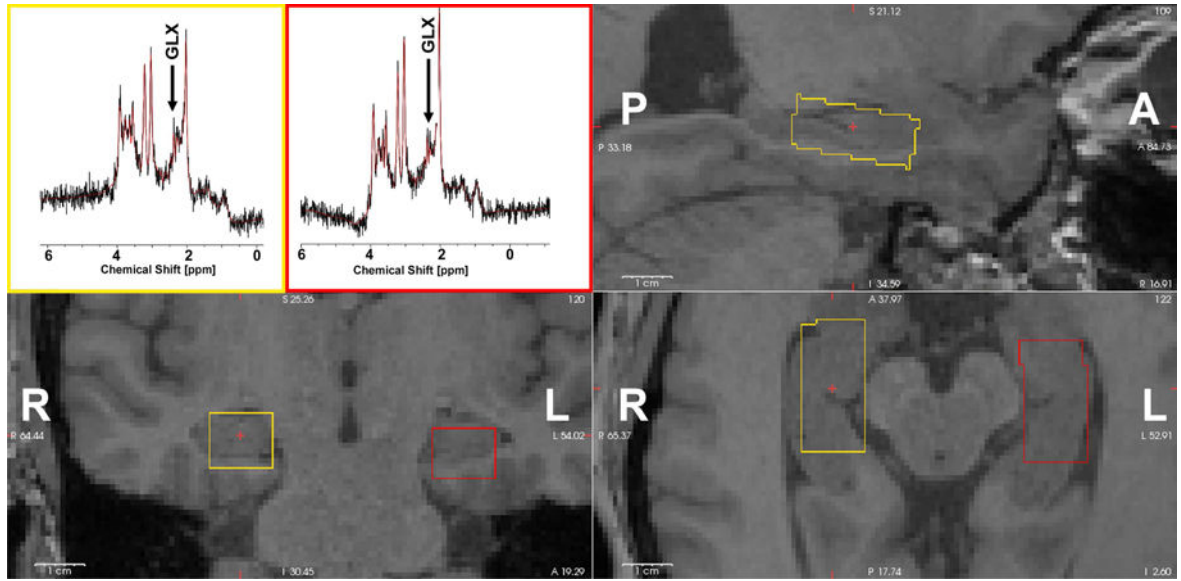


Figure 1. Representative spectra and voxel locations from one participant. Left hippocampus voxel is shown on axial, sagittal and coronal view (yellow). The right hippocampal MRS voxel is shown in red. A-anterior, P-posterior, L-left, R-right.

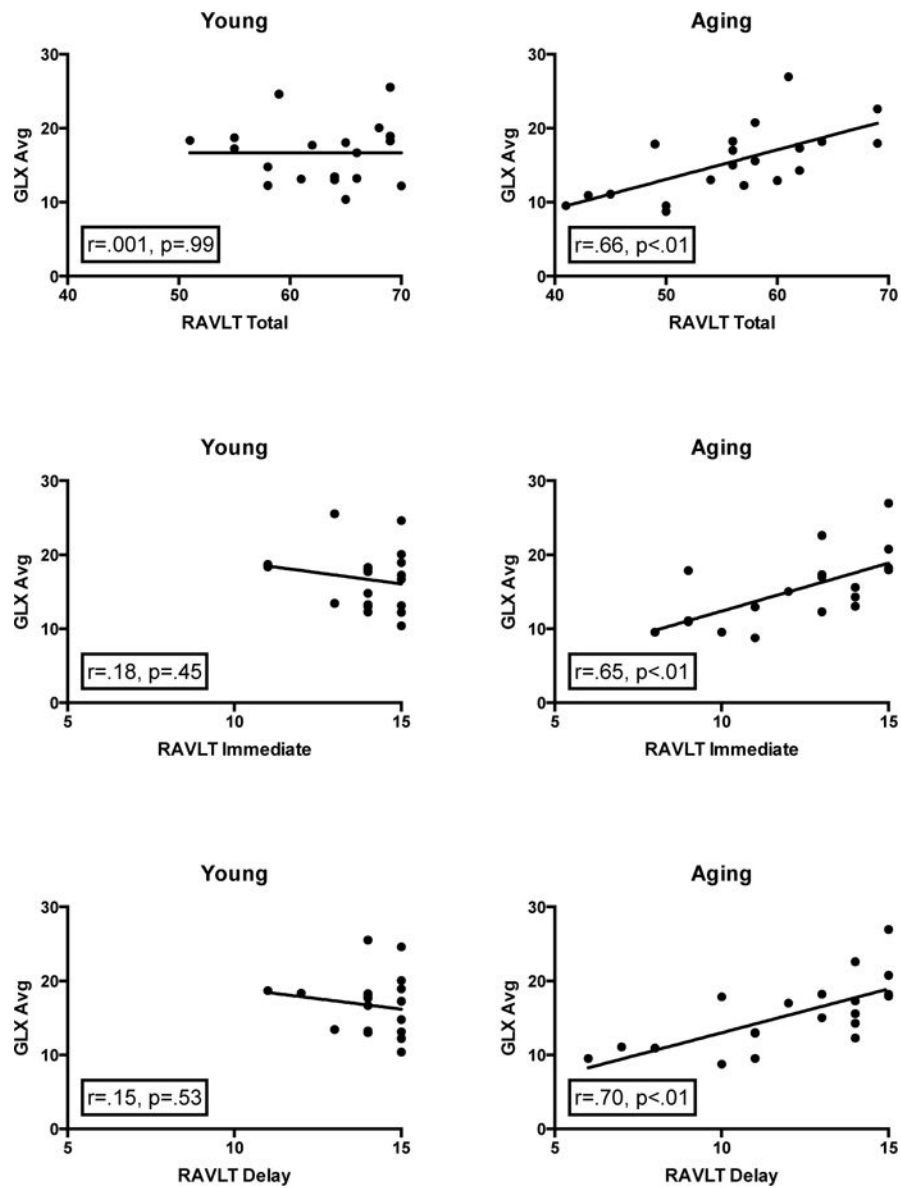


Figure 2. Regression analyses of the average Glx with RAVLT Total Acquisition, Immediate Recall, and Delay Recall memory scores for young and aging participants. The aging group demonstrated a positive relationship between average Glx and each memory measure, yet there is no evidence of this relationship in the young group.

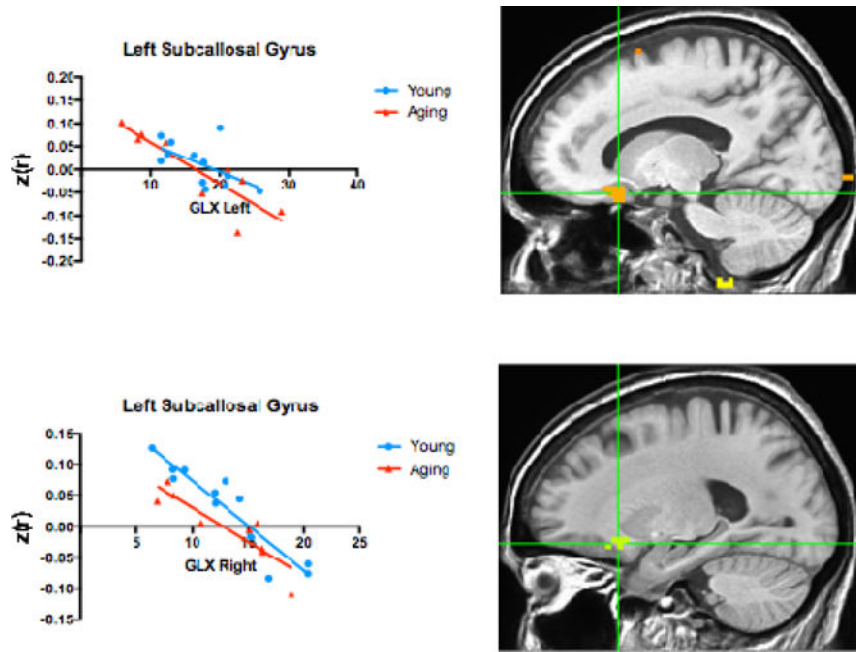


Figure 3. Two regions in the left subcallosal gyrus replicate the negative relationship observed by Wagner et al. (2016) between Glx intensity and RSFC to the right and left anterior hippocampus. The young and aging groups are plotted separately here, but were analyzed as a single group, irrespective of age.

TABLE 1

Age-group contrasts for RAVLT performance, Glx values, brain volumes with values reported as mean (standard deviation).

	Young	Aging	Independent t-test
Age [years]	27 (5)	70 (6)	
Sex [M,F]	8, 11	8, 12	
<i>Behavior</i>			
RAVLT Total	63 (1.3)	56 (1.8)	t(37) = 3.14, p<.01
RAVLT Immediate	13.9 (0.3)	12.4 (0.5)	t(38) = 2.62, p<.05
RAVLT Delay	14.1 (0.3)	12.1 (0.6)	t(38) = 3.01, p<.01
RAVLT Interference	7.1 (2.0)	5.6 (2.0)	t(37) = 2.30, p<.05
<i>Metabolites</i>			
Left Glx	18.4 (4.8)	16.7 (7.4)	t(37) = 0.67, p=.51
Right Glx	14.9 (5.6)	14.0 (4.0)	t(37) = 0.63, p=.53
<i>Volumes</i>			
Left Hippocampus	0.28% (0.02%)	0.25% (0.03%)	t(37) = 3.15, p<.01
Right Hippocampus	0.27% (0.02%)	0.24% (0.04%)	t(37) = 2.71, p<.05
Ventricular Volume	1.0% (0.3%)	2.1% (1.1%)	t(37) = 4.54, p<.01
Left GM fraction	70% (3%)	71% (4%)	t(37) = 0.99, p=.32
Right GM fraction	69% (4%)	69% (4%)	t(37) = 0.71, p=.48
Left WM fraction	22% (4%)	22% (4%)	t(37) = 0.32, p=.75
Right WM fraction	26% (4%)	25% (4%)	t(37) = 0.37, p=.71
Left CSF fraction	8% (2%)	7% (2%)	t(37) = 1.11, p=.27
Right CSF fraction	6% (2%)	5% (2%)	t(37) = 0.61, p=.55
Left Voxel Volume [c.c.]	3.7 (0.2)	3.1 (0.6)	t(37) = 3.48, p<.01
Right Voxel Volume [c.c.]	3.7 (0.2)	3.2 (0.6)	t(37) = 3.05, p<.01
% of Left Voxel = Hipp	59% (6%)	61% (8%)	t(37) = 1.16, p=.25
% of Right Voxel = Hipp	58% (4%)	59% (8%)	t(37) = 0.32, p=.75
Left %GM = Hipp	84% (9%)	86% (8%)	t(37) = 0.77, p=.44
Right %GM = Hipp	85% (6%)	84% (9%)	t(37) = 0.24, p=.81
Avg. MNI Left Voxel [X]	-26 (1)	-27 (1)	
Avg. MNI Left Voxel [Y]	-20 (4)	-19 (2)	
Avg. MNI Left Voxel [Z]	-18 (2)	-20 (6)	
Avg. MNI Right Voxel [X]	27 (2)	26 (1)	
Avg. MNI Right Voxel [Y]	-19 (3)	-19 (2)	
Avg. MNI Right Voxel [Z]	-18 (2)	-19 (2)	

Regions that show a relationship between Glx intensity and RSFC with the left and right hippocampus as seed regions. Region names are defined as the focal point in the Tailairach-Tournoux Atlas. Glx refers to the left or right hippocampal seed that showed RSFC to that region. X (right/left), Y (anterior/posterior) and Z (inferior/superior) coordinates given in Tailairach space (TLRC).

TABLE 2

Region Name	Glx	X	Y	Z	Dir.
Left Subcallosal Gyrus (24)	Left	-13	19	-12	-
Left Subcallosal Gyrus (7)	Right	-20	18	-9	-
Left Superior Frontal Gyrus (11)	Right	-4	16	55	-
Right Middle Frontal Gyrus (8)	Left	28	24	38	-
Left Medial Frontal Gyrus (20)	Right	-4	0	54	+
Left Postcentral Gyrus (3)	Right	-40	-11	32	-
Right Angular Gyrus (36)	Left	37	-58	31	-
Left Cuneus (19)	Right	-4	-77	17	-
Left Inferior Parietal Lobe (5)	Right	-55	-35	26	-
Right Lingual Gyrus (6)	Right	6	-62	-3	-
Left Precuneus (5)	Left	-37	-74	36	-
Right Lingual Gyrus (1)	Right	11	-86	-26	+
Right Inferior Occipital (10)	Right	22	-89	0	+
Right Inferior Occipital Gyrus (15)	Right	32	-82	-3	+
Right Supramarginal Gyrus (8)	Right	38	-45	24	+
Right Precentral Gyrus (9)	Right	57	-14	26	-
Right Middle Temporal Gyrus (18)	Right	50	-52	6	-
Right Cerebellum (29)	Left	6	-60	-27	+
Right Posterior Cingulate (34)	Left	13	-54	11	-
Left Parahippocampal Gyrus (16)	Right	-27	-56	2	-