rijksuniversiteit groningen

Glutamaat-glutamine in rusttoestand in het frontopariëtale netwerk en werkgeheugenprestatie voor patiënten met een milde cognitieve stoornis

RESTING-STATE GLUTAMATE-GLUTAMINE IN THE FRONTOPARIETAL NETWORK AND WORKING MEMORY PERFORMANCE IN PATIENTS WITH MILD COGNITIVE IMPAIRMENT

Masterthese Klinische Neuropsychologie

Adam A. van Rheenen

2361574
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Afdeling Psychologie
Rijksuniversiteit Groningen
Supervisor / Examinator: Professor Branislava Ćurčić-Blake
Tweede beoordelaar: Professor André Aleman

ABSTRACT

Introduction

Up to 50% of patients with amnestic Mild Cognitive Impairment (aMCI) develop full Alzheimer dementia within 30 months. An increasing amount of research suggests that poor working memory (WM) performance predicts progression from aMCI to AD. The WM process has been attributed to the frontoparietal brain network. We measured glutamate-glutamine (Glx) concentrations in two regions of the frontoparietal network and investigated associations with WM performance and memory performance.

Methods

We used the data of 11 patients with MCI. They had participated in the Cogmax-study, a double-blind, randomized trial comparing the effect of synchronous transcranial alternating current stimulation (tACS) and sham stimulation on brain synchronization and cognitive performance. Glx concentration (measured with ^{1}H functional magnetic resonance spectrometry, ^{1}H-fMRS), WM performance (measured with WAIS – digit span test) and memory performance (measured with RAVLT) were evaluated at study baseline and after 10 days of stimulation. The outcome measure of interest was the change in Glx concentration between these timepoints and was analysed with a repeated measures ANOVA, as well as the association between Glx concentration and WM performance and between Glx concentration and memory performance. Additionally, we inspected whether having a memory impairment, based on RAVLT scores, was associated with lower Glx concentrations at baseline.

Results and conclusion

Our findings show that Glx concentrations in the left dorsolateral prefrontal cortex (DLPFC), but not in the left parietal lobe (PL), are associated with memory impairment. We found that WM performance and memory performance differed numerically but not significantly across baseline and post-stimulation measurements. We found that changes in WM performance and memory performance, across baseline and post-stimulation measurements, were not associated with a change in Glx concentration. This indicated that, using the current small sample size (n = 11), no within patient associations could be found between Glx concentrations and WM/memory performance, but between patient association between baseline Glx concentrations and memory impairment were found.

INTRODUCTION

Patients with Mild Cognitive Impairment (MCI) show a decline in cognitive performance that is not explained by increase in age (Peterson et al., 1999). When memory loss is the predominant symptom, as is the case in amnestic MCI (aMCI), almost 50% of the cases will progress to Alzheimer dementia (AD) within 30 months (Petersen et al., 2014; Fischer et al., 2007).

Working memory (WM) may also be affected in patients with MCI (Belleville et al., 2007) and this could hinder the execution of daily activities (Aretouli & Brandt, 2010). Early recognition of WM deficits could be of importance to allow interventions that ameliorate progression to AD (Kirova et al., 2015).

Lesion studies suggested that WM is localized in the prefrontal cortex (D'Esposito & Postle, 2015). Later research, however, found no evidence for WM storage being exclusively localized in a specific brain area (Christophel et al., 2017). Instead, it was suggested that WM is probably a network of various brain areas. Functional neuroimaging studies during WM tasks in healthy individuals identified the frontoparietal network as the mechanism behind WM (Owen et al., 2005). The frontoparietal network includes the dorsolateral prefrontal cortex (DLPFC), the anterior cingulate cortex and parts of the parietal lobe (PL). While the DLPFC is often seen as the more executive part of the frontoparietal network (Kim et al., 2015), required for integrating and manipulating information, the specific role of the parietal regions is more ambiguous (Jonides et al., 1998).

Nonetheless, the search for a chemical substrate that explains WM dysfunction is ongoing. During neuronal activity energy is consumed by means of glucose oxidation, which is directly proportional to glutamatergic neurotransmission (Rothman et al. 1999). In vivo glutamate concentrations can be quantified in a non-invasive manner with functional

magnetic resonance spectroscopy (fMRS). fMRS may help to understand the changes in metabolites during the execution of WM tasks. Healthy individuals show a rise in glutamate levels in the dorsolateral prefrontal cortex during the execution of WM tasks (Woodcock et al., 2018), whereas patients with MCI do not show this increase (Vijayakumari et al., 2020). This difference may reflect a lack of increased metabolic activity and lack of excitatory neurotransmission in patients with MCI.

Additionally, glutamate levels in the medial prefrontal–thalamus–hippocampus network may also play a role in memory performance (Thielen et al., 2018). Patients with AD, which is characterized by memory loss, have lower glutamate concentrations in the bilateral posterior cingulate gyrus than patients with MCI and healthy controls (Fayed et al., 2011), suggesting involvement of glutamate levels in neuronal loss and in turn memory loss.

In this study among patients with MCI, we acquired ^{1}H-fMRS data from two brain regions in the frontoparietal network, relevant to WM, the left dorsolateral prefrontal cortex (left DLPFC) and the left parietal lobe (left PL), [1] to measure resting-state glutamate concentrations, [2] to investigate associations with memory impairment and [3] to investigate within-subject associations (over two time points) with WM performance and memory performance.

METHODS

Study design

This study is an analysis of unpublished data from the ongoing Cogmax-study (www.cogmax.nl). The Cogmax-study is a single center, double-blind, randomized trial comparing the effect of fixed frequency transcranial alternating current stimulation tACS (6 Hz) with individually adjusted frequency tACS and a sham stimulation on cognitive test performance and brain synchronization (box 1). The trial is conducted in accordance with the principle of the Declaration of Helsinki [59th version, October 2008] and the Dutch Medical Research Involving Human Subjects Act. The Medical Ethical Committee of the University Medical Center Groningen (UMCG) approved the study protocol. All participants gave informed consent prior to randomization. Since within-trial unblinding was not allowed, the author of the current sub study was unaware of which condition (fixed frequency tACS, individually adjusted frequency tACS or sham) individual participants were assigned to.

Setting

The Cogmax-study started recruiting participants in September 2018. All experiments were conducted at the University Medical Center Groningen (UMCG) in the Netherlands. fMRI was done at the department of radiology, whereas the other experiments were done at the Neuroimaging Center.

Participants

Participants were recruited from the memory clinic of the UMCG, through general practitioners or hospitals in the area, or through advertisements on the Alzheimer Nederland website (www.alzheimer-nederland.nl), the North-Dutch memory research network (NoNe-GON) or through posters at public places. The diagnosis MCI was based on

the recommendation of the attending neurologist (age ≥50 years, MRI compatible). We excluded patients who had a history of psychiatric or neurological illness other than MCI, metal implants (as MRI is contraindicated for such patients), tattoos containing iron oxide (often found in red pigments), severe scalp lesions or color blindness. Left-handedness, claustrofobia, alcohol or drug abuse or refusal to be informed of structural brain abnormalities that could be detected using MRI during the experiment were other reasons to be excluded. Participants were told not to use alcohol within 2 days prior to the MRI scan. Participants were rewarded € 100 for a total of 13 study visits and were reimbursed for travel costs. For the purpose of this sub study the anonymized and blinded data of 14 participants of the Cogmax study were available.

Box 1 | Synopsis of the umbrella Cogmax-study

Overall aim: To improve executive functioning in patients with aMCI to delay progression of aMCI towards dementia and improve brain synchronization.

Therapeutic intervention: Transcranial alternating current stimulation (tACS) in the theta range, which may alter brain rhythms that target working memory (Herrmann et al., 2013).

Background: Although study outcomes were not univocal, studies observed a positive effect on working memory performance in healthy adults (Hoy et al., 2015) and in patients with MCI (Naro et al., 2016). It is hypothesized that consecutive sessions of tACS further strengthens this effect and delays progression to AD. Müller et al. (2015) observed that elderly subjects, who were exposed to tACS over the parieto-occipital sulcus on five consecutive days, had improved performance on a visual attention task compared to baseline.

Refining stimulation conditions: The application of tACS in patients with MCI has not yet been fully explored. In the Cogmax-study fixed frequency tACS (6 Hz, Polania et al., 2012) is compared with an individually adjusted frequency (Vosskuhl et al., 2015) and a sham stimulation.

Variables

A description of the umbrella Cogmax study can be found in box 2. For the current sub study we used scores on the WAIS - WM tasks (DST forward and backward) and Rey Auditory Verbal Learning Test (RAVLT) (immediate reproduction and delayed reproduction), collected on day 1 and 11, and the ^{1}H-fMRS data obtained during fMRI sessions on day 2 and 11 (figure 1).

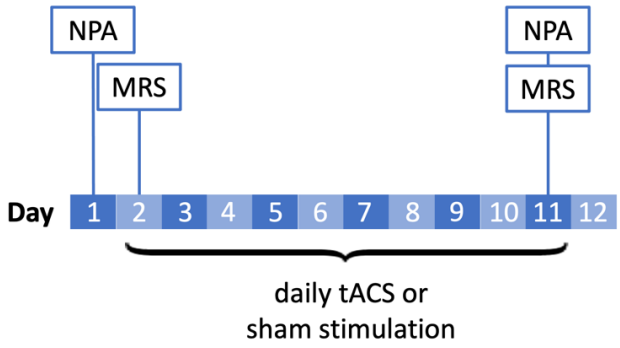

Figure 1 | Schedule of assessments

NPA: fMRS: functional magnetic resonance spectrometry; neuropsychological assessment; tACS: transcranial alternating current stimulation

Neuropsychological assessment

WM performance was assessed with the Wechsler Adult Intelligence Scale (Wechsler, 1981) – digit span test (WAIS-DST). The forward and backward DST are subtests of the WAIS. Participants were orally presented numbers (1-9) in different orders and had to repeat the series forward and backward respectively. Series started with 2 numbers and increased progressively in length until the subject made two consecutive mistakes. While the digit span is defined as the maximum length at which the participant is able to repeat one out of two series, the raw test score equals the sum of correct answers before the test is cut off.

Box 2 | Synopsis of the umbrella Cogmax study part 2: Methods

Test sessions: Participants complete a total of 13 sessions. During the initiation visit as well as on session 11, 1-month follow-up and 1-year follow-up participants undergo a test battery.

Test battery: Resting-state EEG (to measure at what individual frequency there is synchrony with the brain); N-back working memory task using numbers; Vienna reaction time task (VRTT); instrumental activity of daily living (IADL); functional activity questionnaire (FAQ); mini mental state exam (MMSE); face-name associative memory test (FNAME); geriatric depression scale (GDS-30); clinical dementia rating (CDR9); Amsterdamse korte termijn geheugentest (AKTG) – short version; and a neuropsychological evaluation. This includes the RAVLT (immediate and delayed recall; 4 different versions); WAIS-DST: Forward and Backward; Verbal fluency tasks (semantic and letter fluency); Trail making test (TMT); Stroop test; Symbol digit substitution test; Key search (BADS); Wisconsin card sorting test (computer version).

Brain imaging: On day 2 and 11 participants undergo fMRI-sessions during a working memory task and during resting-state. A T1 scan is used to assess anatomical features, consisting of: diffusion tensor imaging (DTI), magnetic transfer imaging (MTI) and functional magnetic resonance spectroscopy (fMRS).

EEG is recorded for approximately 10 minutes (day 2, 11, 1-month follow up and 1-year follow up).

Treatment: For tACS, participants are fitted with an EEG cap on which two electrodes are placed: on the left DLPFC and PL respectfully – at the F3 and P3 positions (10/20 system). Consequently, participants receive stimulation, during which they perform a 2LDT and a WAIS-DST forward and backward, alternately. During sham or active tACS participants perform a two-letter delayed task (2LDT) and the WAIS-DST forward and backward task. These tasks alternate between sessions.

The Rey Auditory Verbal Learning Test (RAVLT, Schmidt, 1996) was designed to test the nature and severity of memory dysfunction by investigating verbal memory. In short, a list of 15 words was repeated 5 times to participant, with direction to recall as much as possible between each repetition. After 20 minutes of "interference", the participant was asked to recall the words. The raw score of the immediate reproduction consists of the sum of words reproduced in all 5 rounds and the raw score of the delayed reproduction consists of only the amount of words reproduced after 20 minutes. T-scores of the RAVLT (immediate and delayed reproduction) were used to classify participants as having memory loss (conform-aMCI) or having intact memory (non-conform-aMCI). It should be noted that this is not a diagnosis but merely a classification based on one neuropsychological test.

Raw scores of the WAIS-DST forward and backward and the RAVLT immediate reproduction and delayed reproduction were transformed into T-scores corrected for gender, age and education level using the Advanced Neuropsychological Diagnostics Infrastructure (ANDI) database (De Vent et al., 2016; Rentergem et al., 2017; Rentergem et al., 2018).

Magnetic resonance spectrometry

We used a 3 Tesla Siemens MAGNETOM MRI scanner (Siemen Healthineers, Munich, Germany) equipped with a 64-channel head coil. Since glutamate cannot be accurately distinguished from glutamine using this type of MRI scanner, the sum of glutamate and glutamine (Glx) is used in the current paper. ^{1}H-fMRS scanning was performed and Point Resolved Spectroscopy (PRESS) images were acquired to measure resting-state Glx concentration (rs-Glx concentration) in two 12 cm^3 voxels (20 × 30 × 20 mm), respectively in the left PL (P3) and the left DLPFC (F3) (figure 2), over a duration of 6 minutes per voxel location, with a 90° flip angle. A selective 123 Hz radio frequency pulse was used. The

acquisition parameters for the spectra included: TE = 35 ms, TR = 2000 ms, bandwidth = 1200, signal averages (NSA) = 160. A T1 image with illustrated voxel position was used as a guideline for placing the voxel (figure 3).

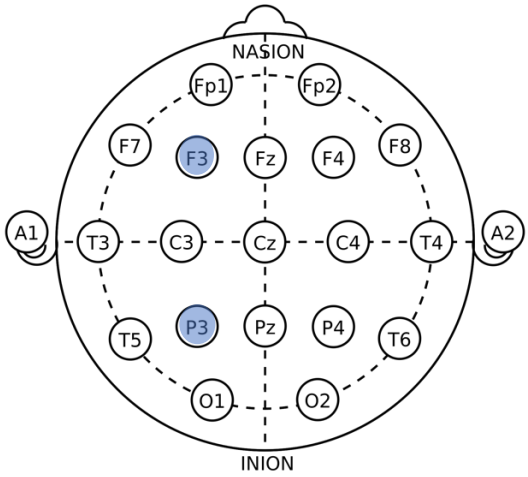

Figure 2 | Approximate position of the voxels at F3 and P3

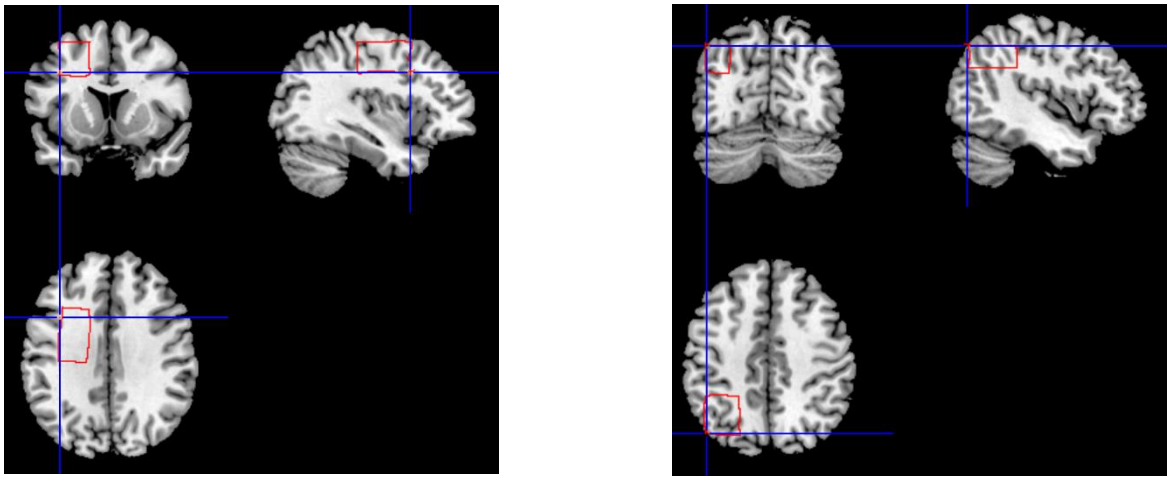

Figure 3 | Illustrated guideline of the voxel placement in the left dorsolateral prefrontal cortex (left) and the left parietal lobe (right)

Outcome measure

The change in rs-Glx concentration between baseline and 10 days post-stimulation for both the P3 and F3-voxel was considered as the main outcome measure of interest. The full width at half-maximum (FWHM) in Hz and signal-to-noise ratio (SNR) were other outcome measures of interest. LCModel, a user independent frequency domain-fitting program, was used to analyse fMRS-spectra and quantify rs-Glx concentrations in the left DLPFC and the left PL (figure 4). The suppressed water peak was used to determine absolute Glx levels. Glx concentration was expressed in parts per million (ppm).

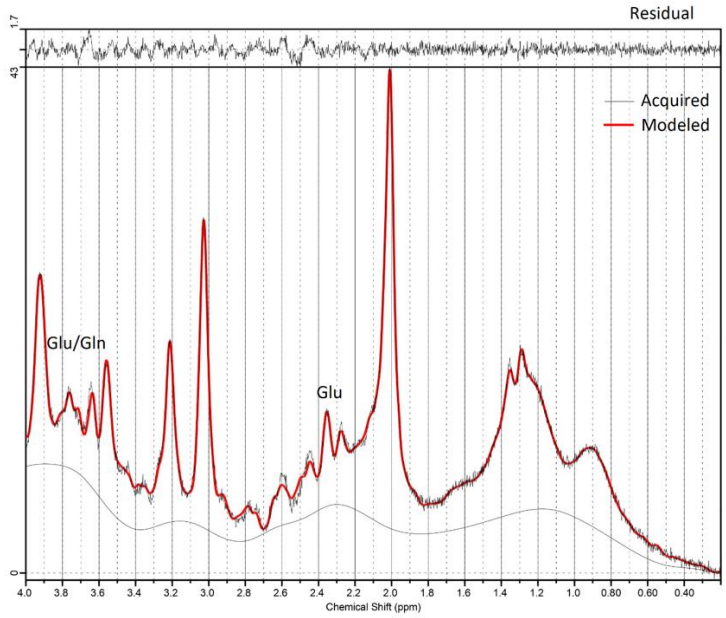

Figure 4 | Example of LCModel output

A ^{1}H-fMRS spectrum from our sample.

Abbreviations: Glu, glutamate; Glu/Gln, glutamate/glutamine; ppm, parts per million

Statistical methods

The FWHM and SNR were used to check the quality of the ^{1}H-fMRS-data. Data were excluded when the FWHM was higher than 30, based on the Siemens spectroscopy manual (Siemens Healthineers, 2012). One-way repeated measures ANOVA was used to check if the means of the FWHM and SNR did not vary significantly across baseline and post-stimulation measurements.

Correlational analyses were used to examine associations between rs-Glx concentrations (at F3 and P3) and aMCI-conform RAVLT T-scores at baseline.

One-way repeated measures ANOVAs were used to assess systematic change between baseline and post-stimulation concentration of rs-Glx at F3 and P3. One-way repeated measures ANOVAs were also used to examine whether time has an effect on performance on the WAIS-DST forward and backward T-scores or the RAVLT immediate reproduction and delayed reproduction T-scores. In case of a systematic improvement across the whole sample, this could imply a possible learning effect. Possible associations between WM or memory performance and baseline and post-stimulation concentration of rs-Glx at F3 and P3 were evaluated with a one-way repeated measures ANOVA. Possible interactions with WAIS-DST (forward and backward) and in RAVLT (immediate reproduction and delayed reproduction) T-scores were checked individually. In case of significant results, the Holm-Bonferroni method was used as a post hoc correction.

All data were analysed electronically using SPSS version 26.0 for Windows (IBM Corp., New York, NY; formerly SPSS Inc., Chicago, IL).

RESULTS

A complete data set was available for 12 Cogmax participants with MCI. After inspecting the data visually, participant 011 was excluded from further analyses of the voxel at P3 (but not for analyses of the voxel at F3) as the baseline rs-Glx concentration at P3 was more than 9 standard deviations above the mean of the other participants (figure 5). For P3 a total of 11 participants was used for the current sub study. Their ^{1}H-fMRS data were considered to be of good quality. The mean age of the participants was 75 years old (± 7.6 years), and 9 of 12 participants (75%) were male. Their education level varied from "finished primary school" to "finished a university degree", with 4 out of 12 participants having "finished a degree in university of applied sciences". The demographic variables are included in table 1.

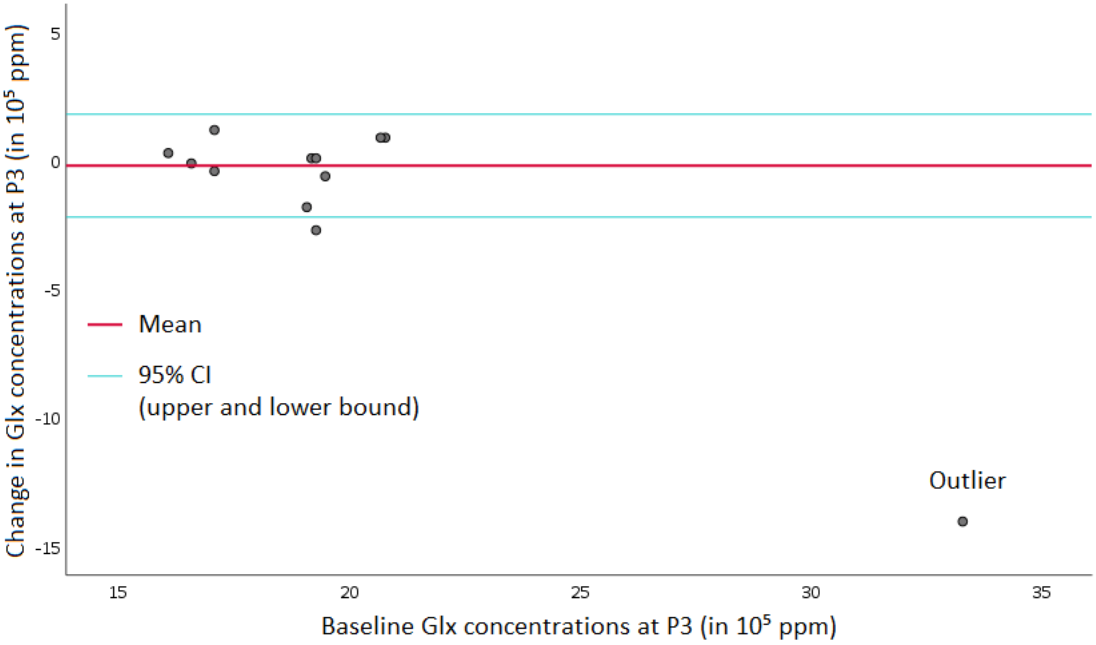

Figure 5 | Scatterplot of rs-Glx concentrations at P3

Modified Bland-Altman plot, with baseline rs-Glx concentrations on the horizontal axis and post-stimulation minus baseline rs-Glx concentrations on the vertical axis.

P3 refers to the voxel-position at the left parietal lobe on the 10/20 system.

Abbreviations: CI, confidence interval; ppm, parts per million; rs-Glx, resting-state glutamate+glutamine

Table 1 | Demographics

Variables	
Mean age in years (SD)	75 (7.6)
Male gender (%)	75
Median education level* (range)	6 (2-7)

Abbreviations: SD, standard deviation

*according to the Verhage scale ranging from 1: unfinished primary school to 7: university degree

Table 2 | WAIS-DST and RAVLT test results at baseline and post-stimulation (n=12)

	WAIS-DST				RAVLT				
	forward		backward		Immediate		Delayed		
Participant	Pre	Post	Pre	Post	Pre	Post	Pre	Post	aMCI
004	46	54	60	60	36	37	19	28	1
008	69	82	72	62	67	57	43	55	0
010	46	57	54	58	38	37	4	4	1
011	45	67	44	54	38	23	7	15	1
013	31	37	27	48	22	28	11	11	1
015	67	56	45	59	31	41	28	52	1
016	59	59	68	64	58	58	42	51	0
017	28	28	26	26	35	30	28	24	1
018	57	46	47	47	28	32	36	22	1
019	36	41	47	42	29	38	30	43	1
020	41	58	43	43	29	39	40	51	0
021	58	71	52	59	36	34	39	35	0

The column at the far right reflects the presence of conform-aMCI, based on baseline RAVLT T-scores.

Abbreviations: WAIS-DST, WAIS digit span test; RAVLT, Rey auditory verbal learning test

Baseline WAIS-DST and RAVLT T-scores indicated that 4 out of 12 participants (33%) did not classify as aMCI patients (table 2). LCModel Fit characteristic at the P3 and F3-voxel were not different over time (table 3).

Table 3 | LCModel fit characteristics at baseline and post-stimulation (F3: n=12; P3: n=11)

	Baseline	**Post-stimulation**	**P values**
FWHM ± SD at F3 (in Hz)	5.0 ± 0.7	5.6 ± 1.6	0.16
FWHM ± SD at P3 (in Hz)	4.8 ± 1.1	4.8 ± 1.0	0.70
SNR ± SD at F3	59.1 ± 23.6	50.4 ± 7.4	0.26
SNR ± SD at P3	52.2 ± 24.5	43.2 ± 7.9	0.37

Mean ± 1 SD FWHM and SNR are described for baseline and post-stimulation, including P values of one-way repeated measures ANOVA across baseline and post-stimulation measurements. F3 and P3 refer to voxel-positions on the 10/20 system.

Abbreviations: FWHM, full width at half-maximum; Hz, Hertz; SNR, signal-to-noise ratio; SD, standard deviation

^{1}H-fMRS data were tested for normality using the Shapiro-Wilk test and normal QQ plots, which confirmed a normal distribution.

Figure 6 shows that participants whose RAVLT scores indicated aMCI had lower baseline Glx concentrations than participants who were classified as non-aMCI. The Glx concentrations in the F3 voxel were respectively 16.3 ± 1.8 and 18.8 ± 0.5 * 10^5 ppm [Pearson correlation = -0.65, P-value = 0.02] and in the P3 voxel 18.0 ± 1.7 and 19.7 ± 0.7 * 10^5 ppm [Pearson correlation=-0.50, P-value = 0.11.

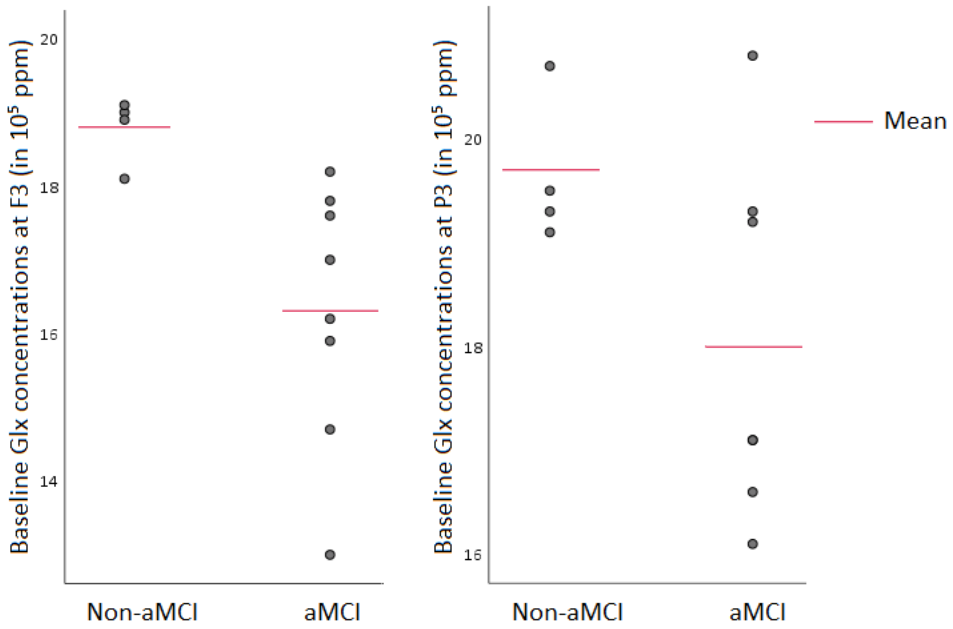

Figure 6 | Baseline resting state Glx concentrations at F3 and P3 in participants classified as non-aMCI and aMCI according the their RAVLT T-scores

There was no significant change in rs-Glx concentrations across baseline and post-stimulation both at the F3 and P3 voxels (table 4). WAIS-DST (forward and backward) and RAVLT T-scores (immediate and delayed reproduction) showed numerically higher values during post-stimulation measurements, but these differences were not significant.

Table 4 | Baseline and post-stimulation resting state Glx, WAIS-DST and RAVLT (F3: n=12; P3: n=11)

Variables		**Baseline**	**Post-stimulation**	**P value**
rs-Glx concentration (in 10^5 ppm)	F3	17.1 ± 1.9	16.9 ± 2.5	0.54
	P3	18.6 ± 1.6	18.4 ± 2.0	0.60
WAIS-DST (T-score)	forward	48.6 ± 13.5	54.7 ± 15.0	0.06
	backward	48.8 ± 14.0	51.8 ± 11.0	0.24
RAVLT (T-score)	immediate reproduction	37.3 ± 12.9	37.8 ± 10.5	0.80
	delayed reproduction	27.3 ± 13.9	32.6 ± 17.8	0.10

One-way repeated measures ANOVAs across baseline and post-stimulation measurements. F3 and P3 refer to voxel-positions on the 10/20 system.

Abbreviations: RAVL, Rey auditory verbal learning test; rs-Glx, resting-state glutamate+glutamine; WAIS-DST, WAIS digit span test

Baseline and post-stimulation rs-Glx concentrations at F3 and P3, corrected for the change of WAIS-DST and the RAVLT performance, were not significantly different (table 5). Since none of the tests yielded significant results, the Holm-Bonferroni method was deemed redundant.

Table 5 | Baseline and post-stimulation rs-Glx concentration, corrected for the change (post-stimulation minus baseline) in performance on the WAIS-DST and the RAVLT

Rs-Glx change	WAIS-DST T scores (change)				RAVLT T scores (change)			
	forward		backward		immediate reproduction		delayed reproduction	
	F score (df)	P value	F score (df)	P value	F score (df)	P value	F score (df)	P value
rs-Glx change F3	0.28 (1,10)	0.61	0.01 (1,10)	0.92	1.66 (1,10)	0.23	<0.01 (1,10)	0.95
rs-Glx change P3	0.52 (1,9)	0.49	0.13 (1,9)	0.73	0.07 (1,9)	0.83	0.51 (1,9)	0.49

One-way repeated measures ANOVAs across baseline and post-stimulation measurements. F3 and P3 refer to voxel-positions on the 10/20 system.

Abbreviations: df, degrees of freedom; RAVL, Rey auditory verbal learning test; rs-Glx, resting-state glutamate+glutamine; WAIS-DST, WAIS digit span test

DISCUSSION

We measured rs-Glx concentrations in participants who were screened by a neurologist for MCI and investigated associations with memory impairments and within-subject associations (across baseline and post-stimulation) with WM performance and memory performance. We found that participants who were classified as conform-aMCI based on the RAVLT had lower baseline rs-Glx concentrations in the left DLPFC than participants who were classified as non-conform-aMCI. No significant correlation between a memory impairment and rs-Glx concentrations was found in the left PL for our sample. Mean post-stimulation WAIS-DST and RAVLT T-scores were numerically (but not significantly) higher. A learning effect could therefore not be demonstrated (Tao, 2019). Since parallel versions were used, a learning effect was not to be expected for the RAVLT. On the other hand, study participants practiced various types of WM tasks between baseline and post-stimulation evaluation. A transfer effect can therefore still take place (Freund, 2007).

Our findings indicated no relationship between the change in WM or memory performance and the change in rs-Glx concentration in the left DLPFC, as well as in the left PL.

Comparison with other studies

A research group from Kerala in India found that people with MCI have lower glutamate levels in the left DLPFC than healthy controls. Moreover, MCI patients did not show an increase in glutamate levels during WM tasks as opposed to healthy controls (Vijayakumari et al., 2020). The limited research on glutamate or Glx concentrations in the left PL has not found a significant correlation with having a memory impairment (Targosz-Gajniak et al., 2013).

Associations between glutamate and WM have been observed in patients with schizophrenia (Kaminski et al., 2018) and in healthy controls (Woodcock, 2018). We were not able to replicate their findings in MCI patients. Vijayakumari et al. (2020), on the other hand, found that glutamate levels remained unchanged during and after a WM task.

It was feasible to measure rs-Glx concentrations using a 3 Tesla Phillips Intera MRI scanner equipped with a 32-channel head coil, however the current sub study was not suitable to evaluate within-subject associations (across baseline and post-stimulation) with WM performance and memory performance.

Limitations

The failure to find significant changes between baseline and post-stimulation test results and associations between rs-Glx concentrations in the left DLPFC and the left PL and WM and memory performance could be inherent to the small sample size. Variations in Glx concentrations, especially in one-and-a-half-week time, are likely to be subtle, and therefore more visible in larger cohorts.

We measured Glx concentrations at resting-state, which may yield different results than when Glx concentrations are measured during WM tasks, as is done in the study by Woodcock (2018).

4 out of 11 participants had intact memory based on baseline RAVLT T-scores. The RAVLT is a sensitive neuropsychological test for aMCI (Estevez-Gonzalez, 2003). It is important to check if these individuals fulfil the aMCI criteria, as the umbrella COGMAX study aims to delay conversion to dementia by applying tACS.

In the COGMAX-study, participants are randomly assigned to 3 conditions. It is hypothesised that participants receiving tACS at fixed frequency tACS (6 Hz) and participants receiving tACS at individually adjusted frequency will have better neuropsychological test-results and higher Glx concentrations than participants who receive a sham stimulation. In the current sub study, however, group assignment was not unblinded. Some participants may have had increased test performance or Glx concentrations due to the tACS intervention.

For this sub study we did not calculate gray and white matter segmentation for ^{1}H-fMRS signals, which may lead to loss of precision of Glx concentration estimates (Tal et al., 2012).

The education level of the current sample is higher than that of the general Dutch population. Most participants in our sample have finished their degree in university or university of applied sciences (64%), whereas in the general Dutch population this would be 30% (CBS, 2017).

Recommendations for future studies

Notwithstanding the absence of significant associations between Glx concentrations and WM and memory performance in this small sub study, the large COGMAX study may be able to demonstrate an association between Glx concentration and WM. Future studies that aim to examine associations between glutamate and WM performance should probably use in-scanner WM tests during the acquisition of ^{1}H-fMRS data, for conclusive evidence. Furthermore, gray and white matter segmentation should be calculated for the voxel's 1H-fMRS signal, as it is said to drastically increase precision.

Conclusions

In the current sub study we measured rs-Glx concentrations. We found that Glx concentrations in the left DLPFC, but not in the left PL, are associated with memory impairment as measured with the RAVLT. Furthermore, in our sample we found that Glx concentrations (in both brain areas), WM performance and memory performance did not significantly differ across baseline and post-stimulation measurements. Lastly, we found that changes in WM performance and memory performance, across baseline and post-stimulation measurements, were not associated with a change in Glx concentration.

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