The secret behind peripheral inflammation and depression: The hypothalamus

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Biological Sciences - Article

Keywords: hypothalamus, hypothalamic-pituitary-adrenals, glutamate metabolites, depression, inflammation

Posted Date: August 11th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-53606/v1>

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Abstract

In recent years there has been increasing evidence of an inflammatory component due to overstimulation of the hypothalamic-pituitary-adrenals (HPA) in depression. The glutamate metabolites (glutamate and glutamine) are important metabolites that are involved in this stimulation. The aim of this study was to determine the concentrations of hypothalamic glutamate metabolites in depression and to investigate their relationship to peripheral inflammation. Participants with diagnosed depression (DE; $n = 24$) and control subjects without depression (HC; $n = 25$) were investigated. Hypothalamic glutamate metabolites were recorded using in vivo magnetic resonance spectroscopy. Peripheral cytokines (IL-6, TNF- α , IFN- γ and IL-1 β) were assessed using Enzyme-Linked Immunosorbent Assay. For statistical analysis, generalized mixed models were computed using Poisson distributions and a log link function. The results show overall higher hypothalamic glutamate metabolites in DE compared to HC. High TNF- α and IL-1 β concentrations are associated with high hypothalamic glutamate metabolites in DE. These results provide initial evidence that, in depression, increased HPA axis activity is associated with peripheral inflammation favored by hypothalamic glutamate metabolites.

Introduction

The hypothalamus is involved in the regulation of many physical and psychological processes, including human homeostasis^{1,2} along the hypothalamic-pituitary-adrenal axis (HPA). The HPA axis regulates hormone and immune functions and plays an important role in inflammation physiology³. The role of the HPA axis in regulating stress responses and their effects on mood and emotions has been well described^{4,5}. In recent years, the importance of the HPA axis for the pathophysiology of various mood disorders such as depression^{6–8} has been emphasized. Models that support the influence of the HPA axis on depression postulate chronically dysregulated activation of the HPA axis, which is also associated with dysregulation of immune function^{8–11}. Various animal studies have shown the relationship between depression and inflammation^{12–15}. In a human study, Pedraz-Petrozzi recently showed correlations between the severity of depression, assessed with the BDI-FS¹⁶, and the pro-inflammatory cytokine TNF- α in people with depression. Overactivation of the HPA axis is also known to affect immune function and cortisol metabolism, ultimately leading to symptoms associated with depression^{17,18}.

Human studies based on the relationship between metabolism and brain activity are required to confirm the hypothesis of overactivation of the HPA axis in depression. In recent years, there have been many suggestions related to imaging and metabolic research for depression. One of these is the examination of brain endophenotypes for depression using in vivo magnetic resonance spectroscopy (MRS)^{19,20}.

Magnetic resonance spectroscopy enables the non-invasive detection of the concentrations of various brain metabolites using strong magnetic fields²¹. The concentrations of metabolites or neurotransmitters can be determined using MRS²², including glutamine metabolites such as glutamate (Glu) and glutamine (Gln)²³ or the metabolite Glx, which is the sum of Gln and Glu²⁴. Glutamate metabolites are mainly associated with central nervous system (CNS) excitatory activity^{23,24}. It is known that glutamic activity in the CNS plays a fundamental role in brain metabolic activity, glucose uptake, and excitatory electrical activity in the CNS¹⁹. Regarding MRS research, the number of publications on glutamate metabolites in depression has increased recently. The brain regions targeted related mainly to cognition and emotion²⁵, such as the hippocampus^{26,27}, the anterior cingulate gyrus^{28,29} and the prefrontal cortex³⁰. Despite a significant number of studies on MRS in depression, there are few studies that involve the hypothalamus or the HPA axis. Only one study found differences between Glx levels in depression³¹. However, this study included multiple sclerosis patients with depression, but not primarily depressed participants without comorbid illness. Aside from this study, there is no study that evaluates hypothalamic activity in depressed patients without other comorbid conditions using MRS.

To further elucidate the role of the HPA axis in depression, the present study focuses on two goals. The first goal is to determine the differences in the concentration of 1H-MRS hypothalamic glutamine metabolites (Glx, Glu and Gln) in people with depression and community healthy controls. The second goal involves the investigation of the relationship between proinflammatory cytokine concentrations in peripheral plasma (IL-6, TNF- α , IFN- γ and IL-1 β) and concentrations of hypothalamic glutamine metabolites (Glx, Glu, and Gln) in patients with depression.

Results

General sample description

General sample description, including baseline clinical and laboratory characteristics of the 49 participants included in the study are listed in table 1. Descriptive data regarding hypothalamic metabolites are also represented in table 1.

¹H-MRS hypothalamic glutamate metabolites: group differences.

The first issue was whether there was a difference between the groups in the hypothalamic metabolites. The main effect of *group* indicated group differences ($\chi^2_{(df=1)} = 24.26, p < 0.001, OR = 2.24, CI95 [1.62; 3.08]$). There was no interaction *metabolite x group* (DE, HC) in the model ($\chi^2_{(df=2)} = 2.79, p = 0.25$), which indicates an overall effect of the *group* on the metabolites. Confounding variables showed effects for *age* ($\chi^2_{(df=2)} = 4.20, p = 0.04$), for *BMI* ($\chi^2_{(df=2)} = 7.23, p = 0.007$), and a negligible effect for *gender* ($\chi^2_{(df=2)} = 2.98, p = 0.08$). The results of this group comparison are shown in figure 1.

Association between the peripheral pro-inflammatory cytokines and ¹H-MRS hypothalamic glutamate metabolites

The second issue was to determine whether there were significant associations between the measured proinflammatory peripheral cytokines and the glutamate hypothalamic metabolites. Pro-inflammatory cytokine concentrations were classified as *low* or *high* by using the median split method. The focus was on the main effects of the *cytokine* (*low, high*) and the interaction effects of the *cytokine x group*. Corresponding odds ratio, confidence intervals and *p*-values are listed in table 2 and are shown in figure 2.

Discussion

First goal - Group differences with regard to 1H-MRS hypothalamic glutamate metabolites: The analysis revealed a group effect, but no interaction effect (*metabolite x group*). This means that participants with depression overall had higher hypothalamic glutamate metabolites than the participants without depression.

To the best of our knowledge, this study is the first to examine the concentration of hypothalamic glutamine metabolites (Glx, Gln and Glu) in depression with no medical comorbid condition. A study by Kantorová et al. studied Glx levels in patients with multiple sclerosis (MS) and healthy subjects³¹, pointing out that Glx levels in MS patients correlated positively with indicators of depression. These results are similar to the results of the current study, which show that patients with primary depression showed higher concentrations of hypothalamic glutamate metabolites than the healthy controls. A recently published meta-analysis of 49 studies showed reduced Glx concentrations in depressed patients only in the medial prefrontal cortex (mPFC)³⁰. However, the hypothalamus was not considered in this study. It can be concluded that different metabolic activation patterns can occur due to depression, since glutamate metabolites are the most important excitatory neurotransmitters in the CNS. In the present study, hypothalamic activation of all glutamate metabolites was associated with depression.

While this is, as far as we know, the first study to report elevated levels of hypothalamic glutamate metabolites in patients with depression, there are several MRS-based studies in depression reporting changes in glutamate metabolites for other CNS regions. However, the relevant literature remains ambiguous, indicating that depression can have different metabolic activation patterns and excitatory activities. On the one hand, there is the study of *Walter et al.*, which found that the Gln levels in the pregenital anterior cingulate cortex (pgACC) of patients with "melancholic" major depression disorder (MDD) were significantly reduced compared to a group of healthy controls³². Similar results have been reported by *Block et al.* who found a significant reduction in the Gln concentration in the hippocampus in patients with MDD³³. On the other hand, the study by Bhagwagar et al. reported increased Glu and Gln concentrations in the occipital cortex in participants with MDD^{34,35}.

The overall higher concentrations of hypothalamic glutamate metabolites found in the present study show that there are no negative changes in neuronal activity such as apoptosis. In various diseases such as hepatic encephalopathy, a higher and sustained increase in a glutamate metabolite (Glu) is observed in the CNS³⁶. In such a case, increased levels of Glu cause apoptosis of the neurons, which leads to cognitive and consciousness disorders³⁶. The overall effect on the three hypothalamic glutamate metabolites could also suggest that overexpressed astrocyte activity in the hypothalamus may be associated with depression.

Astrocytes are part of the glutamate metabolism in the CNS by capturing the glutamate produced by the neurons and converting it to glutamine³⁷. In addition to the intervention of astrocytes in the glutamate-glutamine metabolism, astrocytes also support neurons with energy and metabolism. In this case, glutamine is an important source of energy for neurons^{38,39}. The energy consumption associated with depolarization and excitation in the neurons (glutamate) promotes an immediate glutamine metabolism and, like glucose, supports the neuronal energy requirement^{38,39}. This effect could explain the overall effect on the hypothalamic glutamate metabolites in depression, suggesting that the hypothalamus of depressed people has a higher level of energy, metabolism, and nerve activity. This means that an overreaction of astrocytes could correspond to an overactivation of some neurons, which at the same time would correspond to a stronger excitation (glutamate, neurons) and a stronger cell metabolism (glutamine, astrocytes) in the hypothalamus of depressed patients. Further studies in this direction are still missing to confirm such a proposal.

Second goal - Effects of the MRS glutamate metabolites and the peripheral plasma pro-inflammatory cytokine concentrations between DE and HC: The results of this study showed that people with depression had positive associations between high TNF- α concentrations and Glx/tCr, between high IL-1 β concentrations and Glx/tCr and between high IL-1 β concentrations and Gln/tCr. The effects of possible confounding factors such as age, BMI and gender were taken into account.

The positive relationship between hypothalamic glutamine metabolites and high peripheral proinflammatory cytokine levels in patients with depression but without additional disorders is particularly interesting because few published studies are available to support this correlation. Gonçalves-de-Rezende et al. reported correlations between the cortisol awakening response and Glx concentrations²⁹ in patients with postpartum depression, indicating a positive relationship between hypothalamic activity and pro-inflammatory metabolites (e.g. cortisol). Another study on the relationship between glutamate brain metabolites and peripheral inflammation is the work of Haroon and colleagues⁴⁰. This study did not specifically examine the hypothalamus, but rather changes in cortical and subcortical glutamate metabolites and their relationship to pro-inflammatory markers (e.g. TNF) and depression symptoms during IFN- α treatment in patients with hepatitis C. The results showed a relationship between inflammation markers increased glutamate concentrations and reduced motivation⁴⁰.

Another study on this topic is the work of Slavich and colleagues, which showed stronger associations between activity in the dorsal anterior cingulate cortex (dACC) and in the anterior insula with an increase in the TNF- α -soluble receptor 2⁴¹. These brain regions were also associated with negative affect and distress. The differences between the study by Slavich and colleagues and the present study concern the sample (healthy young adults), the imaging methods and the examined areas of the brain (dACC and anterior insula).

The results of this study showed no positive associations between high IL-6 or IFN- γ concentrations and glutamine hypothalamic metabolites in patients with depression. In addition, TNF- α did not correlate with the individual Gln or Glu concentrations, but with the combined Gln and Glu concentrations, i.e. the Glx concentration. This indicates a synergistic effect of Gln and Glu, which leads to an increase in the variance when the two substances are combined. TNF- α could therefore affect both substances more directly than either. For example, TNF- α could have a direct impact on the metabolites involved in neuronal metabolism and energy support (Gln), as well as excitatory transmission and toxicity (Glu). However, further evidence is needed to understand the effect of TNF- α on hypothalamic and brain metabolism, cell energy processes and excitatory transmission in the human CNS. Finally, in patients with depression, higher IL-1 β levels were associated with both Glx and Gln levels. This indicates that the increase in IL-1 β in people with depression depends more on

hypothalamic neuron metabolism and energy support (Gln) than on excitatory neuronal activity (Glu). The IL-1 family has been shown to induce the activity of various neurotransmitters in HPA ⁴². The induction of various neurotransmitters due to the presence of cytokines from the IL-1 family is mainly related to the activation of cortisol metabolism. The latter is usually associated with depression symptoms and other mood disorders ⁴². In contrast to the current data situation, the results of the present study showed that HPA activity in depression is not due to excitatory neural activity, but mainly to an increased cell metabolism (glutamine) in the HPA axis. However, further studies (i.e. the role of IL-1 β on the hypothalamus in depression) are needed to confirm these results.

Limitations: The current study has shown interesting results regarding hypothalamic glutamate-glutamine metabolites, inflammatory cytokines and depression. However, some aspects should be considered when evaluating the result. As almost always, a larger sample could improve the generalizability of the study results. However, the estimated power (1- β) for a total sample size of 49 participants is 0.98, which is above the accepted minimum power threshold of 1- β = 0.80. This indicates that the sample size for this study design is sufficient to achieve the study goals.

More women than men were included in the study. However, the ratio of women to men was the same in both groups examined and the ratio of female to male participants corresponds to the population of people with depression in Germany ^{43,44}.

Attention should be drawn to a limitation by the method for non-invasive detection of glutamate metabolites, MRS with chemical shift imaging (CSI). Although all extracted voxels come from a brain slice that is selected to best contain the hypothalamus, there is a theoretical possibility that the signals attributed to the hypothalamus may also come from other neighboring structures (i.e. thalamus, corpus callosum, hippocampus, etc.). As far as we know, the method is currently the best for detecting non-invasive hypothalamic glutamate levels.

Future directions: The study also raises other questions that can seriously contribute to understanding the relationship between depression, inflammation, and hypothalamic activity. (1) The investigation of the above mentioned possible synergistic effect of Gln and Glu with TNF- α , (2) the correlation between quinolinic acid from CSF with glutamate-glutamine metabolites and inflammation parameters, (3) investigation of patients with early onset depression in a longitudinal study or cohort to monitor changes in inflammation and glutamate-glutamine metabolites in the CNS over time to investigate the possibility that inflammatory cytokines affect the hypothalamic structure and function of depression, or (4) the study of other MRS metabolites in the CNS in the context of inflammation, HPA axis and depression, for instance energy metabolites. Other inflammatory agents such as IL-17 or IL-34 would also be of interest, (5) as well as the effects of ACTH and cortisol on the glutamate-glutamine metabolites in the hypothalamus.

Conclusions: In summary, this study shows hypothalamic glutamate-glutamine differences between people with depression and people without depression. A positive correlation between the pro-inflammatory cytokine TNF- α and the Glx concentration was found in depression. However, there was no significant correlation between TNF- α and Glu or Gln concentrations. This could show that Gln and Glu play a synergistic role in the relationship with TNF- α , and could indicate that the higher the TNF- α concentration, the greater the simultaneous effects on neuronal metabolism (i.e. Gln) and on excitatory neurotransmission (i.e. Glu). Finally, IL-1 β positively correlated with Glx and Gln levels in depression, which could indicate the effect of IL-1 β on the metabolism of hypothalamic neurons. With this in mind, future studies are needed to clarify the role of metabolism and hypothalamic function in peripheral systemic anti-inflammatory.

Materials And Methods

Study design and selection criteria

A case-control study was carried out. 25 patients who met the ICD-10 criteria for depression or depressive episodes (DE) and 24 healthy controls (HC, community sample) between the ages of 18 and 65 were included in this study. The participants were between 18 and 65 years old. Both samples matched age and gender. The data was collected between June 2019 and September 2019. A sample description with the data on the pro-inflammatory cytokines and the indicator of the severity of the depression is given in table 1.

Volunteers were included in the DE group who had a depressive episode but no accompanying psychotic episode. People with another comorbid psychiatric illness (i.e., bipolar disorder, personality disorder, adaptive syndrome, or post-traumatic stress disorder) have been included as long as the depressive episode has predominated in the past 6 months. Exclusion criteria for both groups were insufficient knowledge of the German language and somatic restrictions that did not allow participation, especially visual or acoustic restrictions. With the exception of depression or depressive episode among the participants in the DE group, any acute or chronic illness was an exclusion criterion for both groups (e.g. flu-like symptoms, systemic lupus erythematosus, rheumatoid arthritis, cancer, etc.). In addition, volunteers were not included in the study if contraindications to participating in MRI studies were identified (e.g. dental implants, pacemakers, etc.).

Each participant or its legal authorized representative were fully informed about the objectives and procedures of this study and gave his written consent to participate. All procedures complied with the Helsinki Declaration. The approval of the local ethics committee of the medical faculty of the Justus Liebig University (JLU) has been obtained. The study complies with the APA ethical standards.

Data collection Blood sampling

3 mL fasting venous blood samples were collected between 8:00 am and 12:00 pm with EDTA K blood sample tubes (S-Monovette 2.7 mL K3E tube with 1.6 mg EDTA/mL, SARSTEDT AG & Co. KG Nümbrecht, Germany) and then centrifuged at 4°C with 1100 x g for 15 minutes. After centrifugation, 1.5 mL plasma was collected and immediately stored at -20°C. Every 4 weeks, the blood samples were collectively delivered to a university research facility, which was about 30 km away, and stored at -80°C for further use.

Levels of interleukin 6 (IL-6), interleukin 1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α) and interferon-gamma (IFN- γ) were measured using ELISA kits (Quantikine ELISA kits, R&D-Systems Inc., Minneapolis, Minnesota, United States of America) with the minimal detection dose (MDD) of: IL-6 = 0.70 pg/mL, IL-1 β = 1.00 pg/mL, TNF- α = 4.00 pg/mL and IFN- γ = 8.00 pg/mL. Intra- and inter-precision values were < 10%.

Values below the ELISA detection limit was considered to be zero pg/mL and included in the analysis. Maximum plasma cytokine values (indicated by the software as '> Max') were excluded from the analysis.

Cytokine concentrations were calculated using the Tecan Reader and Magellan Reader Software (Tecan Group Ltd., Männedorf, Switzerland). For the parameter calculation, the Marquardt's 4-parameter estimation method was used.

Depression

Depressive episodes (DE) were defined according to the diagnostic criteria of the International Statistical Classification of Diseases and Related Health Problems, 10th version (ICD-10) ⁴⁵. The presence of depressive episodes was diagnosed by clinical experts of the psychiatry department from the University Hospital Giessen and Marburg (location - Giessen).

The Beck Depression Inventory - Fast Screening, German version (BDI-FS) ⁴⁶ was used to assess the severity of depressive symptoms. The BDI-FS delivers values in the range between 0 and 21. The highest value indicates a higher depressiveness. The questionnaire shows good internal consistency (Cronbach's α = 0.84) and convergent validity with the PHQ-9 of $r = 0.67$, including validation based on a representative German sample ($n = 2467$). The manual defines categories as "minimal" (0 to 3 points), "mild" (4 to 8 points), "moderate" (9 to 12 points) and "severe" (13 to 21 points).

MR data acquisition

All participants were examined using a ¹H MRS imaging protocol using a 3.0 T MR scanner (SIEMENS MAGNETOM Prisma®) at the Bender Institute for Neuroimaging (BION) at the Justus Liebig University (Giessen, Germany). The measurements were carried out with a 64-channel head coil. The MRS imaging protocol included a T1-weighted magnetization prepared rapid acquisition gradient echo (MPRAGE) image in sagittal position, a ¹H-MRS localizer protocol for CSI, and a CSI with spin echo sequence (SE) ¹H-MRS. Figure 1 shows the region of interest (ROI) used for this study, with two different orientations: sagittal and diagonal.

MPRAGE parameters were set to TR/TE = 1580 ms/2.3 ms, 176 slices, slice thickness = 0.94 mm, field of view (FoV) = 240 mm x 240 mm, matrix size = 256 x 256 voxels, duration = 4 minutes and 29 seconds. For running the ¹H-MRS localizer, images from the T1-weighted MPRAGE were used to locate the slice containing the hypothalamus (reference structures: thalamus, mammillary bodies, inferior and superior colliculi, pituitary gland and fornix). The parameters were set to TR/TE = 6000 ms/115 ms, 1 slice group 1, 11 slices, FoV = 220 mm x 220 mm, slice thickness = 5 mm, distortion factor = 0%, duration = 1 minute and 14 seconds. The parameters for the CSI with SE ¹H-MRS protocol were set to TR/TE = 1700 ms/30 ms, FoV = 160 mm x 160 mm, voxel size = 10 mm x 10 mm x 15 mm, volume of interest (VOI) = 80 mm x 80 mm, number of signal averaging = 3, duration = 6 minutes and 53 seconds.

Imaging processing

The data obtained from the ¹H-MRS were evaluated by two blinded researchers (a physicist and a neuroimaging scientist). The data analysis was carried out on the selected layer with TARQUIN 4.3.1. This software was selected based on positive reports in the literature ^{47,48}. For the current study, three glutamine metabolites from the water-suppressed CSI were quantified using SE ¹H-MRS data, namely glutamate (Glu), glutamine (Gln) and glutamate + glutamine (Glx). Total creatinine (tCr) values were also calculated and used as a reference signal for the analysis.

The ¹H-MRS data obtained for the hypothalamic region could contain a different number of voxels for each participant. To solve this problem, the voxels with a signal-to-noise ratio (SNR) for the glutamate metabolites and a reference signal (tCr) of more than 3 were selected. Hypothalamic voxels with an SNR less than 3 for these metabolites were discarded. The glutamate metabolite concentrations (Glx, Glu, Gln) were calculated for these selected voxels and divided by a reference metabolite. Total creatinine (tCr) was chosen as the reference metabolite. The methodological considerations for MRS data analysis, in particular for determining the metabolite concentrations, have been published elsewhere in the literature ⁴⁹. Finally, the voxel with the highest concentration was selected for each participant and for each of the metabolites (Glx/tCr, Glu/tCr, Gln/tCr) and fed into the statistical analysis.

Statistical Analysis

The data set in this study consists of the metabolic data of the hypothalamus (Glx/tCr, Gln/tCr, and Glu/tCr) and the plasma peripheral proinflammatory cytokines (IL-6, IL-1 β , TNF- α , and IFN- γ). Both showed positively-skewed, non-Gaussian distributions with a few large values. The TARQUIN software expresses the hypothalamic metabolic data (Glx/tCr, Gln/tCr, and Glu/tCr) in ppm (parts per million), which corresponds to count data with a discrete distribution and is best represented by a Poisson distribution. Therefore, generalized mixed models were calculated using the Poisson distribution with log link-function for Glx/tCr, Glu/tCr, and Gln/tCr as dependent variables. The best model was selected according to the AIC criterion.

Differences in hypothalamic metabolites between DE and HC were investigated with the fixed effects *metabolite* (Glx/tCr, Gln/tCr, Glu/tCr) and *group* (DE, HC). For analyzing differences in hypothalamic metabolites with respect to the pro-inflammatory cytokines, the data from the pro-inflammatory cytokines was dichotomized using the median split method. These dichotomized variables were individually introduced into the model as fixed effects together with the group status (DE, HC). A model was calculated for each combination of a hypothalamic metabolite and a cytokine.

For all models, the variables *age*, *BMI* and *gender* were included as covariates to control the possible effects of these variables. *Smoking behavior* and *medication intake* explained the variance of the models only minimally, so that they were not included in the models.

Models were computed using the R software v. 3.6. (R Core Team, 2019. *R: A Language and environment for statistical computing*) and the toolbox GAMLj (Gallucci, M., 2019. *GAMLj: General analyses for linear models*). This toolbox also is implemented in the software *jamovi* 1.2.5.0 (Jonathon Love, Damian Dropmann, Ravi Selker. *The Jamovi Project*. Retrieved from <https://www.jamovi.org>).

Declarations

This study was supported financially by the German academic exchange program (DAAD) and by the foundation “*Immunität und Seele*” (Prof. Norbert Müller). The authors would like to thank the neuroimaging team of the Bender Institute of Neuroimaging (BION) from the Psychology and Sports Science faculty – Justus Liebig University (Giessen). Finally, the authors would like to thank the team of the Clinical Immunology – Kerckhoff Clinic, specially Ms. Mona Arnold and Ms. Carina Scheiyäck for their contribution to the ELISA-tests assessments.

Conflict of Interests

The authors declare no conflicts of interest.

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Tables

Table 1 – General sample description, baseline clinical and laboratory characteristics, and hypothalamic glutamate metabolites

	Status	Age	BMI	IL-6	TNF- α	IL-1 β	IFN- γ	BDI-FS _{TOT}	Gln/tCr	Glu/tCr	Glx/tCr
N	HC	25	25	25	25	25	25	25	25	25	25
	DE	24	24	24	24	24	24	24	24	24	24
Mean	HC	26.80	24.90	1.72	9.12	1.19	15.90	1.80	2.95	2.13	5.13
	DE	31.90	24.10	2.76	8.82	1.02	17.40	7.25	7.19	3.45	10.40
SD	HC	8.73	5.86	1.34	1.17	0.98	3.11	2.16	7.66	2.46	9.56
	DE	12.80	4.17	2.09	1.40	0.68	10.60	5.17	16.50	6.23	18.20
Skewness	HC	3.42	1.92	1.94	0.34	3.53	0.60	1.76	4.28	1.75	4.10
	DE	1.30	0.66	1.00	1.55	3.74	4.41	0.53	4.13	3.34	3.20
Q1	HC	22.00	21.50	1.03	8.23	0.68	14.20	0.00	< 0.01	< 0.01	1.77
	DE	22.00	21.30	1.20	8.06	0.77	13.70	3.00	0.90	< 0.01	2.13
Median	HC	25.00	22.30	1.38	8.95	0.97	15.40	1.00	0.88	1.78	2.80
	DE	26.50	23.40	2.25	8.59	0.87	15.20	7.00	2.02	0.94	4.70
Q3	HC	29.00	25.20	1.81	9.84	1.11	17.50	3.00	1.87	3.37	3.89
	DE	36.30	27.40	3.43	9.48	1.06	16.70	10.00	4.82	4.67	8.66
IQR	HC	7.00	3.70	0.78	1.61	0.43	3.30	3.00	1.87	3.37	2.12
	DE	14.30	6.10	2.23	1.42	0.29	3.00	7.00	3.92	4.67	6.53
		minimal	mild	moderate	severe						
BDI-FS (Categories)	HC	20	4	1	0						
	DE	9	6	5	4						

Abbreviations: HC = healthy controls group, DE = participants with depression or depressive episodes, BDI – FS = Beck Depression Inventory – Fast Screening; BDI – FS_{TOT} = Total values of the Beck Depression Inventory – Fast Screening; IL-6 = Interleukin 6; TNF- α = Tumor necrosis factor alpha; IL-1 β = Interleukin 1 Beta; IFN- γ = Interferon gamma; Gln/tCr = ¹H-MRS Glutamine concentration; Glu/tCr = ¹H-MRS Glutamate concentration; Glx/tCr = ¹H-MRS Glutamine-glutamate concentration.

Table 2 – Association between peripheral pro-inflammatory cytokines and 1H-MRS hypothalamic glutamate metabolites.

		cytokine (low vs. high)			Group (DE vs. HC) x cytokine (low vs. high)		
		OR	CI95	p	OR	CI95	p
Glx/tCr	TNF- α	1.72	[1.00: 2.95]	0.05*	3.73	[1.35: 10.32]	0.01*
	IL-6	1.53	[0.83: 2.83]	0.18	1.07	[0.38: 3.04]	0.90
	IL-1 β	1.72	[0.91: 3.26]	0.09	3.34	[1.26: 8.82]	0.02*
Gln/tCr	IFN- γ	1.86	[1.15: 3.01]	0.01*	1.70	[0.50: 5.70]	0.39
	TNF- α	1.58	[0.82: 3.03]	0.17	3.93	[0.96: 16.18]	0.06
	IL-6	1.36	[0.63: 2.93]	0.43	1.38	[0.35: 5.40]	0.65
Glu/tCr	IL-1 β	1.16	[0.56: 2.42]	0.69	5.91	[1.61: 21.72]	0.007*
	IFN- γ	3.54	[1.70: 7.37]	< 0.001*	0.94	[0.20: 4.45]	0.94
	TNF- α	2.61	[1.17: 5.84]	0.02*	2.73	[0.82: 9.13]	0.10
Glx/tCr	IL-6	1.77	[0.81: 3.88]	0.16	0.54	[0.16: 1.88]	0.33
	IL-1 β	4.65	[1.99: 10.84]	< 0.001*	1.98	[0.53: 7.34]	0.31
	IFN- γ	0.79	[0.44: 1.44]	0.45	2.44	[0.56: 10.64]	0.23

Abbreviations: HC = healthy controls group, DE = participants with depression or depressive episodes; IL-6 = Interleukin 6; TNF- α = Tumor necrosis factor alpha; IL-1 β = Interleukin 1 Beta; IFN- γ = Interferon gamma; Gln/tCr = ¹H-MRS Glutamine concentration; Glu/tCr = ¹H-MRS Glutamate concentration; Glx/tCr = ¹H-MRS Glutamine-glutamate concentration; OR = Odds ratio; CI95 = 95-percent confidence intervals; p = P-values.

Figures

Figure 1

(a) Region-of-Interest (ROI) of the present study. From left to right (1) sagittal plane with hypothalamus (green) representing TI-MRI, (2) diagonal plane with hypothalamus (green) representing CSI-MRS localizer and (3) the glittered diagonal plane with hypothalamus (green) from the CRI-MRS localizer. (b) Generalized mixed model marginal estimates and 95% confidence intervals for H MRS ratios of Glx/tCr, Gln/tCr, and Glu/tCr, divided into groups DE and HC.

Figure 2

Generalized mixed model marginal estimates and 95% confidence intervals for the H MRS ratios of Glx/tCr, Gln/tCr, and Glu/tCr divided into groups (DE, HC) and cytokine levels (low, high). Corresponding statistics are in listed in Table 2.