Acute stress effects on GABA and glutamate levels in the prefrontal cortex: A 7T ^{1}H magnetic resonance spectroscopy study

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ABSTRACT

There is ample evidence that the inhibitory GABA and the excitatory glutamate system are essential for an adequate response to stress. Both GABAergic and glutamatergic brain circuits modulate hypothalamus-pituitary-adrenal (HPA)-axis activity, and stress in turn affects glutamate and GABA levels in the rodent brain. However, studies examining stress-induced GABA and glutamate levels in the human brain are scarce. Therefore, we investigated the influence of acute psychosocial stress (using the Trier Social Stress Test) on glutamate and GABA levels in the medial prefrontal cortex of 29 healthy male individuals using 7 Tesla proton magnetic resonance spectroscopy. *In vivo* GABA and glutamate levels were measured before and 30 min after exposure to either the stress or the control condition. We found no associations between psychosocial stress or cortisol stress reactivity and changes over time in medial prefrontal glutamate and GABA levels. GABA and glutamate levels over time were significantly correlated in the control condition but not in the stress condition, suggesting that very subtle differential effects of stress on GABA and glutamate across individuals may occur. However, overall, acute psychosocial stress does not appear to affect *in vivo* medial prefrontal GABA and glutamate levels, at least this is not detectable with current practice ^{1}H-MRS.

1. Introduction

Stressful situations require a prompt response of the organism to promote adaptation and survival (McEwen, 2004). Hypothalamus-pituitary-adrenal (HPA) axis functionality is essential for such a response, and depends on many mediators, such as steroid hormones (*e.g.* cortisol), neurotransmitters (including glutamate and GABA), cytokines, and neuropeptides, which all function in time- and brain area-dependent manners (Joëls and Baram, 2009). The hippocampus, amygdala and prefrontal cortex (PFC) are particularly interesting regions, as they project onto the HPA axis *via* the inhibitory GABA and excitatory glutamate system (Ulrich-Lai and Herman, 2009), but the stress-related dynamics of these systems largely remain unclear. Of note, stress exposure generally increases prefrontal cortex glutamate levels in the rodent brain (for review see (Popoli et al., 2012)) and mostly decreases brain GABA levels, depending on the type and duration of stress, and the brain region examined (Acosta and Rubio, 1994; Bedse et al., 2015;

Borsini et al., 1988; de Groote and Linthorst, 2007; Gunn et al., 2011; Otero Losada, 1988; Petty and Sherman, 1981). In addition, rapid changes in GABA(A) receptors occur after acute stress in animals (Skilbeck et al., 2010).

In contrast to the abundance of animal studies examining the relation between stress and GABA/glutamate levels, human studies are scarce. Currently, the only method to directly measure GABA and glutamate levels in the living human brain is proton magnetic resonance spectroscopy (^{1}H-MRS). Using ^{1}H-MRS to detect stress-related differences in metabolite levels in the PFC, one study reported increased glutamate + glutamine levels after chemically induced panic (Zwanzger et al., 2013) and another study showed decreasing GABA levels under threat of shock (Hasler et al., 2010). However, to the best of our knowledge, the influence of acute psychosocial stress on GABA and glutamate levels in the human brain is unknown. Investigating the mechanisms underlying psychosocial stress is relevant in light of the impact of repeated psychosocial stress exposure on the risk for and course of psychiatric disorders (Brenner et al., 2009; Lange et al., 2013).

Recent technical developments at a field strength of 7 Tesla (T) enable improved measurement of *in vivo* glutamate and GABA levels in the human brain (Boer et al., 2011; Mullins et al., 2014). Scanning at higher field strength yields greater spectral dispersion and thereby

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more reliable signal quantification (Govindaraju et al., 2000), which is of particular interest since glutamate and especially GABA are present at low concentrations in the brain (5–15 mmol/kg (Govindaraju et al., 2000) and ±1 mmol/kg (Wijtenburg et al., 2015), respectively).

Therefore, we aimed to investigate acute psychosocial stress-induced changes in glutamate and GABA levels in the human medial PFC (mPFC) as measured with ^{1}H-MRS in a 7T MRI scanner. Based on the available studies in rodents (Drouet et al., 2015; Otero Losada, 1988; Popoli et al., 2012; Skilbeck et al., 2010), we hypothesized that, compared to the control condition, stress would increase glutamate levels and decrease GABA levels in the human mPFC.

2. Material and methods

2.1. Participants

Healthy non-smoking male individuals (age 18–40, $N = 30$) were recruited from the general population in The Netherlands (see Table 1). Participants did not take any medication and had not previously been enrolled in any stress-related research. The absence of mental disorders according to DSM-IV criteria was confirmed using the Mini International Neuropsychiatric Interview (MINI)-plus (Sheehan et al., 1998) conducted by a trained rater. On the day of the test, participants did not take heavy meals or drinks other than water and they abstained from heavy exercise for at least 2 h prior to arrival. Absence of psychoactive substance use (amphetamines, MDMA, barbiturates, cannabinoids, benzodiazepines, cocaine, and opiates) was determined by self-report and verified with a urine multi-drug screening device (InstantView) (Vinkers et al., 2013).

2.2. General

All experimental procedures were approved by the ethical review board of the University Medical Center Utrecht and performed according to the ICH guidelines for Good Clinical Practice and the Declaration of Helsinki. We measured GABA and glutamate levels in the mPFC of participants who were randomized to either the validated stress ($N = 15$) or control ($N = 15$) condition of the Trier Social Stress Test (TSST) (Kirschbaum et al., 1993). During a first visit, participants were familiarized with the 7T MRI scanner environment and scanning procedure with a 15-minute scan session to reduce any potential stressful associations with the scanning environment. Throughout the second visit, participants completed a 120-minute protocol during which GABA and glutamate levels were quantified in the mPFC before (time point 1) and 30 min after (time point 2) exposure to either the stress or the control condition (Fig. 1). Scanning around 30 min after stress exposure (time point 2) was selected to coincide with the cortisol peak of the stress response (Vinkers et al., 2013).

2.3. Stress and control conditions

All experimental conditions were carried out between 2 PM–9 PM to minimize diurnal variations of cortisol secretion. The stress condition was carried out in accordance with previously published methods (Kirschbaum et al., 1993). Five minutes before the stress or control intervention, all participants received written instructions. In the stress
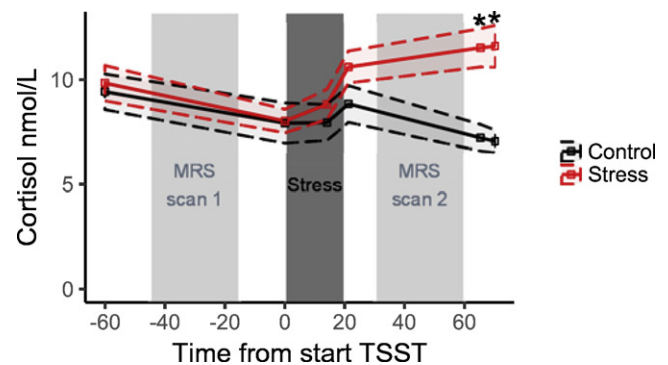
Table 1
Baseline sample characteristics in the total sample and per condition.

Variable	Total (n = 29)	Control (n = 14)	Stress (n = 15)
Mean age in years (SD)	24 (5)	23 (5)	25 (5)
Childhood maltreatment (mean, range)	31 (25–44)	31 (27–39)	32 (25–44)
Major life events (mean, range)	2.5 (0–6)	2.6 (0–5)	2.5 (0–6)
Daily hassles (mean, range)	17.6 (5–44)	16.9 (5–44)	18.5 (6–44)

Fig. 1. Cortisol levels over time before and after exposure to the control condition ($N = 15$) or the stress condition ($N = 14$). The dotted lines represent the standard error. * = p-value < 0.01 (comparing the stress to the control condition in the posthoc test per time point).

condition, participants delivered a public speech and performed a challenging mental arithmetic while being seemingly videotaped and recorded in front of an evaluative panel that did not show any signs of social support. The combination of an evaluated public speech and cognitive task reliably stimulates the HPA axis by integrating uncontrollability with threat to the social self and self-esteem. The control condition consisted of a speech and simple arithmetic without the presence of a video camera or evaluative panel. Thus the control task has a comparable cognitive load without the social evaluative aspects that stimulate the HPA axis (Het et al., 2009). Salivary cortisol levels were measured using six saliva samples (Salivettes) collected over a 120-minute time period (from 60 min prior to the experimental condition up to 60 min afterwards, Fig. 1). Cortisol was measured using an in-house radioimmunoassay as previously published (Vinkers et al., 2013). For three individuals one saliva sample was missing due to insufficient saliva for reliable detection. For these three missing samples (that were all prior to the experimental condition), a value was imputed based on all other cortisol measurements, age and experimental condition. The area under the curve with respect to the increase (AUCi) of cortisol was calculated as previously described (Pruessner et al., 2003). Moreover, the cortisol peak response was calculated representing a more dynamic measure of temporal changes as previously published (5th sample–2nd sample) (Vinkers et al., 2013).

2.4. Magnetic resonance spectroscopy

All scans were performed on a 7T MRI scanner (Philips, Cleveland, OH, USA) with a birdcage transmit head coil driven by two amplifiers in combination with a 32 channel receive coil (Nova Medical, Inc.). A T1-weighted MP-RAGE sequence was acquired for voxel placement (174 slices, TR = 4 ms, TE = 1.8 ms, flip angle = 7°, field of view = 246 × 246 × 174 mm). Glutamate levels were detected in a 20 × 20 × 20 mm^3 voxel using an sLASER sequence (semi-localized by adiabatic selective refocusing; TE = 30–36 ms, TR = 5000 ms, 32 averages, max B1 = 17–20 μT, no OVS (Boer et al., 2011)). The TE was either 30 ms in case we could reach a local B1 of 20 μT, or 36 ms in case the local B1 was between 17 and 20 μT. J-difference spectral editing was used to differentiate the GABA signal from other metabolites. The macromolecular contribution to the GABA signal was minimized by using symmetric editing around the macromolecule resonance at 1.7 ppm, alternating the editing pulse between 1.9 ppm (GABA refocused) and 1.5 ppm (GABA undisturbed) (Andreychenko et al., 2012). GABA-edited ^{1}H-MRS spectra were obtained using a MEGA-sLASER sequence (TE = 74 ms, TR = 4000 ms, 64 averages, no OVS (Andreychenko et al., 2012)) in a 25 × 25 × 25 mm^3 voxel. Non-water suppressed spectra were obtained in order to calculate absolute concentrations of metabolites. Prior to ^{1}H-MRS acquisition, RF shimming on the region of interest was used to optimize phase settings of the individual transmit channels.

Second order B0 shimming was automatically performed before data acquisition. For tissue segmentation purposes, a whole-brain three-dimensional fast field echo T1-weighted scan was obtained (450 slices, slice thickness = 0.8 mm, TR = 7 ms, TE = 3 ms, flip angle = 8°, field of view = 250 × 200 × 180 mm, 312 × 312 acquisition matrix, SENSE factor 2.7, scan duration = 408 s). The voxel was placed in the mPFC with the posterior edge adjacent to the corpus callosum and the anterior edge placed to avoid signal from the cerebrospinal fluid ($25 \times 25 \times 25$ mm^3 voxel for GABA; $20 \times 20 \times 20$ mm^3 voxel for glutamate Fig. 2). To ensure comparable voxel placement before and after the experimental procedure, screenshots of the first scan were used to place the voxel in the second scan session.

2.5. Metabolite quantification

Data from 32 receiver coils were combined after amplitude weighting and phasing based on the water reference signal, and noise decorrelation based on a noise scan. The water reference signal was also used for eddy current correction and as an internal standard for GABA and glutamate quantification. Metabolites (including glutamate) were quantified from conventional MR spectra using LCModel-based software implemented in Matlab ((Provencher, 1993); NMR Wizard) which relies on *a priori* knowledge of spectral components of metabolites. Measured macromolecules and sixteen simulated metabolite profiles were fitted to each spectrum: taurine (Tau), myo-inositol (m-Ino), glutathione (GSH), glutamine (Gln), glutamate (Glu), GABA, *N*-acetyl aspartyl glutamate (NAAG), *N*-acetyl aspartate (NAA), phosphocreatine (PCr), creatine (Cr), phosphoethanolamine (PE), glycerophosphocholine (GPC), phosphocholine (PCh), lactate (Lac), aspartate (Asp) and glycine (Gly). The baseline of the spectral fit was adjusted by incorporating possible lipid and water artifacts. GABA-edited MR spectra were frequency-aligned with the singlet resonance of choline prior to subtraction of odd and even acquisitions. Fitting of the GABA-edited spectra was performed by frequency-domain fitting of the GABA and creatine resonances to Lorentzian line shapes using in-house Matlab tools (Andreychenko et al., 2013).

Spectral fitting was assessed based on (i) visual inspection by two independent investigators and (ii) a Cramer Rao lower bound (CRLB) estimate lower than 10% for GABA and glutamate, which is lower than the generally recommended CRLB of 20% (Provencher, 2015). The CRLB represents estimates of the standard deviations of the fit for each metabolite. Based on these criteria, one MEGA-sLASER scan was excluded. A typical example of metabolite fits has been included in Fig. 2. Due to data transfer problems, GABA data was missing for three individuals and we did not have an anatomical scan to calculate GABA and glutamate concentrations for one individual. Glutamate and GABA data were available for 29 and 26 individuals, respectively.

To correct for partial volume effects in the voxel, grey matter (f_{GM}), white matter (f_{WM}) and CSF(f_{CSF}) fractions per voxel were obtained using segmentation of the anatomical images with statistical parametric mapping software (SPM8) according to the unified segmentation method (Ashburner and Friston, 2005) (see Appendix A, Supplementary Method 1 for full description). In short, the sum value for each of the three tissue masks was divided by the sum of all three tissue masks for each voxel, resulting in $f_{GM} + f_{WM} + f_{CSF} = 1$ (see Appendix A, Supplementary Table 1). Correction for partial volume differences did not change any of the results and we used the corrected values for all analyses (see Appendix A, Supplementary Note 1 for the analyses without partial volume corrections).

2.6. Questionnaires

To investigate possible confounding by childhood maltreatment, life events, and daily hassles on cortisol stress reactivity, participants completed validated self-report questionnaires of childhood trauma (Childhood Trauma Questionnaire (CTQ) (Bernstein et al., 2003)), major life events (Lifetime Stressor Checklist-Revised (LSC-R) (Wolfe et al., 1996)) and current daily hassles (Dutch Everyday Problem Checklist (Vinkers et al., 2014)).

2.7. Statistical analysis

2.7.1. General

All statistical analyses were carried out using R version 3.2.1 (R-Core-Team, 2014). For regression modelling, the Limma package was used (Smyth, 2004). There were no outliers (defined as having a Cook's Distance > 1). Age was included as a covariate to adjust for age variation in brain metabolite levels (Marsman et al., 2013). In all regression models, GABA or glutamate levels after the experimental condition, adjusted for baseline GABA or glutamate levels, were used as primary outcome. Since trauma exposure can influence cortisol stress reactivity, we examined if group differences existed for childhood trauma, major life events or daily hassles.

2.7.2. Stress exposure: effects on GABA and glutamate levels

The main aim of the current study was to investigate the effects of stress on GABA and glutamate levels. Therefore, we examined the association between GABA or glutamate levels after the experimental condition (stress *versus* control) in a linear regression model while adjusting for age and baseline GABA or glutamate levels. We also calculated the correlations between GABA and glutamate concentrations before and after the experimental condition to examine whether these correlations would differ in the stress compared to the control condition.

2.7.3. Stress-induced cortisol levels: effects on GABA and glutamate levels

First we examined whether the cortisol response over time differed between the stress and the control condition using Mixed Model Repeated measures with the nlme package in R. In this model condition,
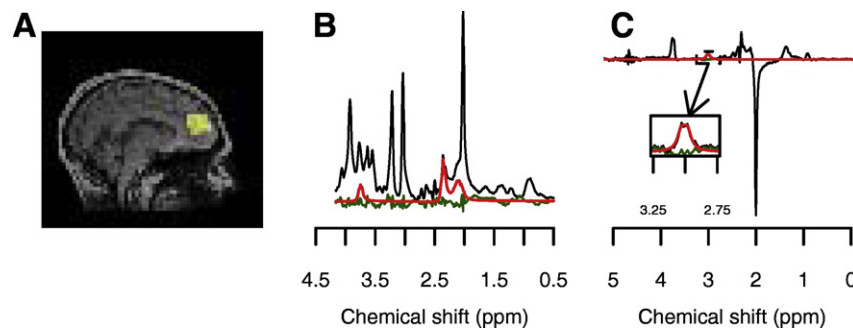

Fig. 2. Representative example of voxel placement (yellow rectangle) in the medial prefrontal cortex (panel A), an sLASER spectrum (panel B) and an edited MEGA-sLASER spectrum (panel C). In the spectra, the red line denotes the individual metabolite fit of respectively glutamate (panel B) or GABA (panel C) and the green line is the residual after fitting the metabolites. Insert: zoom of the GABA peak in the edited MEGA-sLASER spectrum.

time, age and the interaction between time and condition were modeled as fixed effects and we included a by-subject random effect of intercepts and slopes. If a significant interaction was present between the experimental condition and time, the specific time points between the control and stress condition were identified in planned posthoc tests with Bonferroni adjustment for multiple comparisons. Next, we examined the association between cortisol stress reactivity (expressed as $AUCi_{CORTISOL}$ or peak cortisol response) and longitudinal change in GABA or glutamate levels after the experimental condition in a linear regression with age and baseline GABA or glutamate levels as covariates.

2.8. Reliability ^{1}H-MRS measurement

To evaluate the reproducibility of ^{1}H-MRS measurements over time, we calculated the intraclass correlation coefficient (ICC) for GABA and glutamate in the control condition. Consistent with previous neuroimaging studies, an ICC of 0.7 was deemed acceptable (Cai et al., 2012).

3. Results

3.1. Group characteristics

No significant group differences were present for age, baseline GABA or glutamate levels in the mPFC, partial volumes in the mPFC voxels, childhood trauma, major life events and minor stressors (Tables 1 and 2).

3.2. Stress related differences in prefrontal GABA and glutamate levels

Stress did not significantly affect prefrontal GABA and glutamate levels (glutamate B = −0.1 t = −0.2 p = 0.86, model fit: F(3,25) = 0.49 R^2 = 0.06; GABA B = 0.22 t = 1.3 p = 0.20, model fit: F(3,22) = 3.9 R^2 = 0.26) (Fig. 3). Both for GABA and glutamate, the levels before and after the control condition were significantly correlated (GABA r = 0.45, p = 0.03, Glutamate r = 0.43, p = 0.04). In contrast, before-after levels were not significantly correlated in the stress condition (GABA r = −0.09 p = 0.69, Glutamate r = 0.18 p = 0.46).

3.3. Cortisol stress reactivity, GABA and glutamate levels

Cortisol levels over time were significantly higher in the stress condition compared to the control condition (Condition × Time interaction F(4,112) = 9.89, p < 0.001). Posthoc tests indicated higher cortisol levels in the stress condition at the time points immediately after the second ^{1}H-MRS measurement (t_{65min} B = 4.6 p = 0.002 and t_{70min} B = 4.8 p < 0.001) (Fig. 1). As expected, stress exposure resulted in a larger cortisol peak response (B = 3.9 t = 3.2 p = 0.003, model fit: F(2,27) = 7.0 R^2 = 0.29) and a trend towards a higher $AUCi_{CORTISOL}$ (B = 149 t = 2.04 p = 0.05, model fit: F(2,27) = 2.1 R^2 = 0.07). However, cortisol release was not associated with changes in either glutamate ($AUCi_{CORTISOL}$ B = 4.7×10^{-04} t = −0.3 p = 0.73, model fit: F(3,25) = 0.52 R^2 = −0.05; cortisol increase B = −0.02 t = −0.3, p = 0.79, model fit: F(3,25) = 0.5 R^2 = −0.06) or GABA levels ($AUCi_{CORTISOL}$ B = 3.4×10^{-05} t = 0.08 p = 0.93, model fit: F(3,22) = 3.1 R^2 = 0.20; cortisol increase B = −0.009 t = −0.3 p = 0.73, model fit: F(3,22) = 3.1 R^2 = 0.20).

3.3.1. Reliability ^{1}H-MRS signal

In the control group the ICC estimates were similar for GABA (ICC = 0.60) and glutamate (ICC = 0.57), but lower than the 0.7 cut-off deemed acceptable in previous neuroimaging studies that aimed to establish reproducibility between scans (Cai et al., 2012).

4. Discussion

In the current study, we investigated the influence of acute psychosocial stress on glutamate and GABA levels in the human prefrontal cortex using 7T ^{1}H-MRS. Stress exposure did not significantly alter GABA and glutamate levels compared to the control condition. Moreover, the peak and AUCi cortisol response were not associated with changes in prefrontal GABA or glutamate levels. Nonetheless, whereas both GABA and glutamate before and after the control condition were significantly correlated, this was not the case in the stress condition, possibly indicating very subtle stress effects differing across individuals.

4.1. GABA and glutamate changes in response to stress

GABAergic and glutamatergic neurotransmission are pivotal for restoring homeostasis after acute stress, with the mPFC and hippocampus constituting two key regions affecting HPA axis activity (Ulrich-Lai and Herman, 2009). Rodent studies indicate increased stress-related prefrontal glutamate levels, primarily based on studies carried out in synaptosomes (for review see (Popoli et al., 2012)). In the hippocampus either no effect (Popoli et al., 2012) or a rapid increase in glutamate levels or release probability was observed (Karst et al., 2005; Venero and Borrell, 1999). Also, several hours after acute stress glutamatergic transmission was found to be enhanced, both in the PFC (Yuen and Yan, 2009; Yuen et al., 2011) and in the hippocampus (Karst and Joëls, 2005). In contrast, acute stress generally decreased frontal and hippocampal GABAergic transmission (Biggio et al., 2007). Some evidence suggests that the direction of GABAergic transmission change after acute stress is stressor dependent, both in the hippocampus (for review see (Linthorst and Reul, 2008)) and in the frontal cortex (Acosta and Rubio, 1994; Bedse et al., 2015).

Although many rodent studies report GABA and glutamate differences after stress, human studies investigating stress-induced GABA and glutamate levels are scarce. In contrast to our findings of no stress-related differences in GABA and glutamate levels after acute psychosocial stress, two previous ^{1}H-MRS studies reported increased glutamate (Zwanzger et al., 2013) and decreased GABA (Hasler et al., 2010) levels in the prefrontal cortex after chemically induced panic and threat of shock, respectively. However, it is important to note several differences in study methodology. First, we used an extensively validated psychosocial stressor with a social evaluative aspect which induces a robust cortisol response (for review see (Foley and Kirschbaum, 2010)). Nevertheless, it is possible that GABA and glutamate levels are not as susceptible to this type of stressor as to chemically induced panic or threat of shock. In addition, since the stress task needs to be carried out outside of the MR scanner, voxel placement, shimming and voxel localization were done twice, which may have led to more within-subject variation. Moreover, while the previously reported glutamate increase was detected 10 min after stress (Zwanzger et al., 2013) and the GABA decrease 15 min after stress (Hasler et al., 2010), we measured
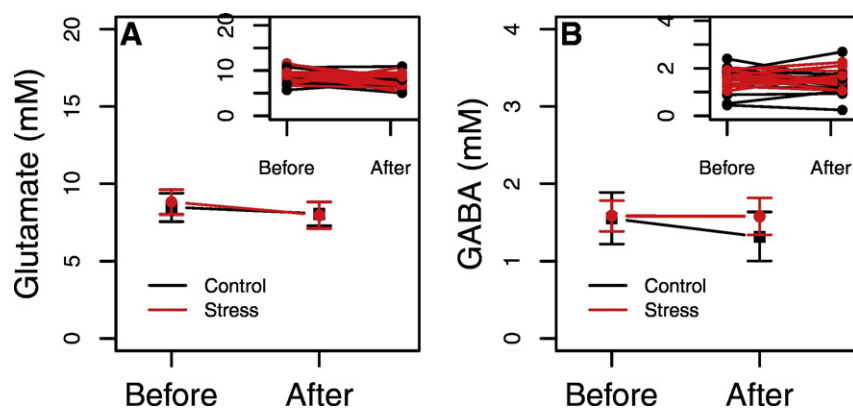
Table 2
Glutamate and GABA levels in the total sample and per condition.

Variable	Total (n = 29)[a]	Control (n = 14)[a]	Stress (n = 15)[a]
Glutamate (mM) before (mean, SD)	8.7 ± 1.5	8.6 ± 1.6	8.8 ± 1.4
Glutamate (mM) after (mean, SD)	8.0 ± 1.4	8.3 ± 1.0	8.0 ± 1.5
GABA (mM) before (mean, SD)	1.6 ± 0.5	1.6 ± 0.6	1.6 ± 0.4
GABA (mM) after (mean, SD)	1.4 ± 0.5	1.3 ± 0.5	1.5 ± 0.4

[a] For GABA total N = 26, stress N = 12 and control N = 14.

Fig. 3. Mean glutamate (A) and GABA (B) levels before and after the task in either the control (black) or stress (red) condition. Error bars indicate the standard error per condition. Insert: individual GABA and glutamate levels for each participant.

GABA and glutamate levels at the peak of the cortisol response (30 min after stress) in line with a bidirectional relationship between cortisol levels and GABA and glutamate (Mody and Maguire, 2012). We cannot exclude that GABA and glutamate levels immediately after stress exposure are more relevant for cortisol stress reactivity than GABA and glutamate levels 30 min after stress. A final difference with previous studies is the use of a 7T scanner enabling better separation of glutamate from glutamine and, in the edited sequence, GABA detection with less macromolecule contamination than at lower field strength. This is particularly relevant as macromolecular content can contribute to > 30% of the GABA signal (Andreychenko et al., 2012; Choi et al., 2010).

4.2. GABA and glutamate in stress-related psychopathology

Notwithstanding the absence of stress or cortisol effects on prefrontal GABA and glutamate levels, adequate functioning of these systems is crucial for maintaining mental health. In support, GABA system abnormalities have been described in a wide range of stress-related disorders, including major depressive disorder (MDD) (Luscher et al., 2011), post-traumatic stress disorder (PTSD) (Geuze et al., 2008), schizophrenia (Gonzalez-Burgos et al., 2015), and general mental health problems after military deployment (Schür et al., 2016). In addition, differences in the glutamatergic system have also been linked to MDD (Luykx et al., 2012), PTSD (Pitman et al., 2012), and schizophrenia (Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014). It remains to be determined to what extend stress-related dynamics of these systems are disturbed in stress-related psychopathology.

4.3. GABA and glutamate quantification

The GABA and glutamate levels we report are in line with the previously reported human brain concentrations of GABA (± 1 mmol/kg (Wijtenburg et al., 2015)) and glutamate (5–15 mmol/kg (Govindaraju et al., 2000)). Direct comparison between our values and those of others is complicated by differences in quantification methodology. Important parameters affecting metabolite concentrations include the quantification software, number of metabolites fitted, partial volumes in the voxel location and MRS data quality checks (Alger, 2010; van de Bank et al., 2015; Mullins et al., 2014; Schür et al., 2016). Our glutamate measurement with the sLASER sequence in the mPFC was less consistent (ICC = 0.57) than previously reported for other brain areas (van de Bank et al., 2015). This lower consistency might be inherent to greater physiological variation in the brain region under study or it could be related to the control task completed in between measurements. Alternatively, it could have resulted from less reliable signal due to magnetic field inhomogeneity, as the region of interest was situated near the paranasal sinuses. Importantly, all Cramer Rao lower bounds (CRLBs) were below 10% which indicates that the measurements were of good quality.

4.4. Conclusion

In conclusion, we did not find a significant effect of acute stress exposure or cortisol stress reactivity on prefrontal GABA and glutamate levels in the human brain. Although GABA and glutamate levels over time were not correlated in the stress condition, possibly indicating very subtle and differential effects of stress on GABA and glutamate across individuals, our findings suggest that a stress effect on GABA and glutamate levels in the medial prefrontal cortex 30 min after psychosocial stress is absent or at least undetectable using current practice ^{1}H-MRS.

Author contributions

All authors have written and approved the manuscript. D.W.K, C.H.V. and L.C.H. designed and collected the data for the study. J.P.W. and V.O.B. helped with the spectroscopy analyses. R.R.S. ran the segmentation analyses. L.C.H. performed the statistical analyses under supervision of C.H.V. and M.P.M.B. R.S.K. and M.J. supervised and commented on the manuscript at all stages.

Competing financial interests

Dr Vinkers, Dr Boks, Dr Klomp, Dr Wijnen, Dr Boer, Mr Schür, Prof. Joëls, Prof. Kahn and Ms Houtepen declare no potential conflict of interest.

Acknowledgments

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.nicl.2017.01.001.

References

Acosta, G.B., Rubio, M.C., 1994. GABAA receptors mediate the changes produced by stress on GABA function and locomotor activity. JOUR. Neurosci. Lett. 176, 29–31.

Alger, J.R., 2010. Quantitative proton magnetic resonance spectroscopy and spectroscopic imaging of the brain: a didactic review. Top. Magn. Reson. Imaging 21:115–128. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3103086&tool=pmcentrez&rendertype=abstract.

Andreychenko, A., Boer, V.O., Arteaga De Castro, C.S., Luijten, P.R., Klomp, D.W.J., 2012. Efficient spectral editing at 7 T: GABA detection with MEGA-sLASER. Magn. Reson. Med. 68, 1018–1025.

Andreychenko, A., Klomp, D.W.J., De Graaf, R.A., Luijten, P.R., Boer, V.O., 2013. In vivo GABA T2 determination with J-refocused echo time extension at 7 T. NMR Biomed. 26, 1596–1601.

Ashburner, J., Friston, K.J., 2005. Unified segmentation. Neuroimage 26, 839–851.

Bedse, G., Romano, A., Tempesta, B., Lavecchia, M.A., Pace, L., Bellomo, A., Duranti, A., Micioni Di Bonaventura, M.V., Cifani, C., Cassano, T., Gaetani, S., 2015. Inhibition of anandamide hydrolysis enhances noradrenergic and GABAergic transmission in the prefrontal cortex and basolateral amygdala of rats subjected to acute swim stress. J. Neurosci. Res. 93, 777–787.

Bernstein, D.P., Stein, J.A., Newcomb, M.D., Walker, E., Pogge, D., Ahluvalia, T., Stokes, J., Handelsman, L., Medrano, M., Desmond, D., Zule, W., 2003. Development and validation of a brief screening version of the Childhood Trauma Questionnaire. Child Abuse Negl. 27, 169–190.

Biggio, G., Concas, A., Follesa, P., Sanna, E., Serra, M., 2007. Stress, ethanol, and neuroactive steroids. Pharmacol. Ther. 116, 140–171.

Boer, V.O., van Lier, A.L.H.M.W., Hoogduin, J.M., Wijnen, J.P., Luijten, P.R., Klomp, D.W.J., 2011. 7-T 1H MRS with adiabatic refocusing at short TE using radiofrequency focusing with a dual-channel volume transmit coil. NMR Biomed. 24, 1038–1046.

Borsini, F., Mancinelli, A., D'Aranno, V., Evangelista, S., Meli, A., 1988. On the role of endogenous GABA in the forced swimming test in rats. Pharmacol. Biochem. Behav. 29: 275–279 (http://www.ncbi.nlm.nih.gov/pubmed/3362921).

Brenner, K., Liu, A., Laplante, D.P., Lupien, S.J., Pruessner, J.C., Ciampi, A., Joober, R., King, S., 2009. Cortisol response to a psychosocial stressor in schizophrenia: blunted, delayed, or normal? JOUR. Psychoneuroendocrinology 34, 859–868.

Cai, K., Pr Nanga, R., Lamprou, L., Schinstine, C., Elliott, M., Hariharan, H., Reddy, R., Epperson, C.N., 2012. The impact of gabapentin administration on brain GABA and glutamate concentrations: a 7 T 1H-MRS study. Neuropsychopharmacology 37, 2764–2771.

Choi, C., Dimitrov, I.E., Douglas, D., Patel, A., Kaiser, L.G., Amezcua, C.A., Maher, E.A., 2010. Improvement of resolution for brain coupled metabolites by optimized 1H MRS at 7 T. NMR Biomed. 23, 1044–1052.

de Groote, L., Linthorst, A.C.E., 2007. Exposure to novelty and forced swimming evoke stressor-dependent changes in extracellular GABA in the rat hippocampus. Neuroscience 148, 794–805.

Drouet, J.B., Fauvelle, F., Maunoir-Regimbal, S., Fidier, N., Maury, R., Peinnequin, A., Denis, J., Buguet, A., Canini, F., 2015. Differences in prefrontal cortex GABA/glutamate ratio after acute restraint stress in rats are associated with specific behavioral and neurobiological patterns. Neuroscience 285, 155–165.

Foley, P., Kirschbaum, C., 2010. Human hypothalamus-pituitary-adrenal axis responses to acute psychosocial stress in laboratory settings. Neurosci. Biobehav. Rev. 35, 91–96.

Geuze, E., van Berckel, B.N.M., Lammertsma, A.A., Boellaard, R., de Kloet, C.S., Vermetten, E., HGM, W., 2008. Reduced GABA benzodiazepine receptor binding in veterans with post-traumatic stress disorder. Mol. Psychiatry 13 (74–83):3 (http://www.ncbi.nlm.nih.gov/pubmed/17667960).

Gonzalez-Burgos, G., Cho, R.Y., Lewis, D.A., 2015. Alterations in cortical network oscillations and parvalbumin neurons in schizophrenia. Biol. Psychiatry 77, 1031–1040.

Govindaraju, V., Young, K., Maudsley, A.A., 2000. Proton NMR chemical shifts and coupling constants for brain metabolites. NMR Biomed. 13, 129–153.

Gunn, B.G., Brown, A.R., Lambert, J.J., Belelli, D., 2011. Neurosteroids and GABA(A) receptor interactions: a focus on stress. Front. Neurosci. 5:131 (http://www.ncbi.nlm.nih.gov/pubmed/22164129).

Hasler, G., van der Veen, J.W., Grillon, C., Drevets, W.C., Shen, J., 2010. Effect of acute psychological stress on prefrontal GABA concentration determined by proton magnetic resonance spectroscopy. Am. J. Psychiatry 167, 1226–1231.

Het, S., Rohleder, N., Schoofs, D., Kirschbaum, C., Wolf, O.T., 2009. Neuroendocrine and psychometric evaluation of a placebo version of the "Trier Social Stress Test". Psychoneuroendocrinology 34, 1075–1086.

Joëls, M., Baram, T.Z., 2009. The neuro-symphony of stress. Nat. Rev. Neurosci. 10, 459–466 (http://www.ncbi.nlm.nih.gov/pubmed/19339973\\nhttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2844123).

Karst, H., Joëls, M., 2005. Corticosterone slowly enhances miniature excitatory postsynaptic current amplitude in mice CA1 hippocampal cells. J. Neurophysiol. 94, 3479–3486.

Karst, H., Berger, S., Turiault, M., Tronche, F., Schutz, G., Joels, M., 2005. Mineralocorticoid receptors are indispensable for nongenomic modulation of hippocampal glutamate transmission by corticosterone. Proc. Natl. Acad. Sci. 102, 19204–19207 (http://www.pnas.org/content/102/52/19204.abstract?ijkey=2aaac46f8cd7f137a9d7e8722032992e4dc653bc&keytype2=tf_ipsecsha\\nhttp://www.pnas.org/cgi/doi/10.1073/pnas.0507572102).

Kirschbaum, C., Pirke, K.M., Hellhammer, D.H., 1993. The "Trier Social Stress Test"—a tool for investigating psychobiological stress responses in a laboratory setting. Neuropsychobiology 28, 76–81.

Lange, C., Zschucke, E., Ising, M., Uhr, M., Bermpohl, F., Adli, M., 2013. Evidence for a normal HPA axis response to psychosocial stress in patients remitted from depression. Psychoneuroendocrinology 38, 2729–2736.

Linthorst, A.C.E., Reul, J.M., 2008. Stress and the brain: solving the puzzle using microdialysis. Pharmacol. Biochem. Behav. 90, 163–173.

Luscher, B., Shen, Q., Sahir, N., 2011. The GABAergic deficit hypothesis of major depressive disorder. Mol. Psychiatry 16:383–406. http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=3412149&tool=pmcentrez&rendertype=abstract.

Luykx, J.J., Laban, K.G., van den Heuvel, M.P., Boks, M.P.M., Mandl, R.C.W., Kahn, R.S., Bakker, S.C., 2012. Region and state specific glutamate downregulation in major depressive disorder: a meta-analysis of 1H-MRS findings. Neurosci. Biobehav. Rev. 36, 198–205.

Marsman, A., Mandl, R.C., Van Den Heuvel, M.P., Boer, V.O., Wijnen, J.P., Klomp, D.W.J., Luijten, P.R., Hulshoff-Pol, H.E., 2013. Glutamate changes in healthy young adulthood. Eur. Neuropsychopharmacol. 23, 1484–1490.

McEwen, B.S., 2004. Protection and damage from acute and chronic stress: allostasis and allostatic overload and relevance to the pathophysiology of psychiatric disorders. Trans. NY Acad. Sci. 1032, 1–7.

Mody, I., Maguire, J., 2012. The reciprocal regulation of stress hormones and GABAA receptors. Front. Cell. Neurosci. 6, 1–6.

Mullins, P.G., McGonigle, D.J., O'Gorman, R.L., Puts, N.A., Vidyasagar, R., Evans, C.J., Edden, R.A., 2014. Current practice in the use of MEGA-PRESS spectroscopy for the detection of GABA. Neuroimage 86, 43–52.

Otero Losada, M.E., 1988. Changes in central GABAergic function following acute and repeated stress. Br. J. Pharmacol. 93, 483–490.

Petty, F., Sherman, A.D., 1981. GABAergic modulation of learned helplessness. Pharmacol. Biochem. Behav. 15, 567–570.

Pitman, R.K., Rasmusson, A.M., Koenen, K.C., Shin, L.M., Orr, S.P., Gilbertson, M.W., Milad, M.R., Liberzon, I., 2012. Biological studies of post-traumatic stress disorder. Nat. Rev. Neurosci. 13:769–787. http://www.nature.com/doifinder/10.1038/nrn3339.

Popoli, M., Yan, Z., McEwen, B.S., Sanacora, G., 2012. The stressed synapse: the impact of stress and glucocorticoids on glutamate transmission. Nat. Rev. Neurosci. 13, 22–37.

Provencher, S.W., 1993. Estimation of metabolite concentrations from localized in vivo proton NMR spectra. Magnes. Res. 30, 672–679.

Provencher, S.W., 2015. LCModel & LCMgui User's Manual. ELEC. s-provencher.com/pages/lcm-manual.shtml. http://s-provencher.com/pub/LCModel/manual/manual.pdf.

Pruessner, J.C., Kirschbaum, C., Meinlschmid, G., Hellhammer, D.H., 2003. Two formulas for computation of the area under the curve represent measures of total hormone concentration versus time-dependent change. Psychoneuroendocrinology 28, 916–931.

R-Core-Team, 2014. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, COMP. Vienna, Austria. http://www.r-project.org/.

Schizophrenia Working Group of the Psychiatric Genomics Consortium, 2014n. Biological insights from 108 schizophrenia-associated genetic loci. Nature 511, 421–427.

Schür, R.R., Boks, M.P., Geuze, E., Prinsen, H.C., Verhoeven-Duif, N.M., Joëls, M., Kahn, R.S., Vermetten, E., Vinkers, C.H., 2016. Development of psychopathology in deployed armed forces in relation to plasma GABA levels. Psychoneuroendocrinology 73, 263–270.

Schür, R.R., Draisma, L.W.R., Wijnen, J.P., Boks, M.P.M., Koevoets, M.G.J.C., Joëls, M., Klomp, D.W.J., Kahn, R.S., Vinkers, C.H., 2016. Brain GABA levels across psychiatric disorders: a systematic literature review and meta-analysis of 1H-MRS studies. Hum. Brain Mapp.:n/a http://dx.doi.org/10.1002/hbm.23244.

Sheehan, D.V., Lecrubier, Y., Sheehan, K.H., Amorim, P., Janavs, J., Weiller, E., Hergueta, T., Baker, R., Dunbar, G.C., 1998. The Mini-International Neuropsychiatric Interview (M.I.N.I.): the development and validation of a structured diagnostic psychiatric interview for DSM-IV and ICD-10. J. Clin. Psychiatry 59 (Suppl. 2), 22–33.

Skilbeck, K.J., Johnston, G.A., Hinton, T., 2010. Stress and GABA receptors. J. Neurochem. 112, 1115–1130.

Smyth, G.K., 2004. Linear models and empirical bayes methods for assessing differential expression in microarray experiments. Stat. Appl. Genet. Mol. Biol. 3 (Article 3).

Ulrich-Lai, Y.M., Herman, J.P., 2009. Neural regulation of endocrine and autonomic stress responses. Nat. Rev. Neurosci. 10, 397–409.

van de Bank, B.L., Emir, U.E., Boer, V.O., van Asten, J.J., Maas, M.C., Wijnen, J.P., Kan, H.E., Oz, G., Klomp, D.W.J., Scheenen, T.W., 2015. Multi-center reproducibility of neurochemical profiles in the human brain at 7 T. NMR Biomed. 28, 306–316.

Venero, C., Borrell, J., 1999. Rapid glucocorticoid effects on excitatory amino acid levels in the hippocampus: a microdialysis study in freely moving rats. Eur. J. Neurosci. 11, 2465–2473.

Vinkers, C.H., Zorn, J.V., Cornelisse, S., Koot, S., Houtepen, L.C., Olivier, B., Verster, J.C., Kahn, R.S., Boks, M.P.M., Kalenscher, T., Joëls, M., 2013. Time-dependent changes in altruistic punishment following stress. Psychoneuroendocrinology 38, 1467–1475.

Vinkers, C.H., Joëls, M., Milaneschi, Y., Kahn, R.S., Penninx, B.W., Boks, M.P.M., 2014. Stress exposure across the life span cumulatively increases depression risk and is moderated by neuroticism. Depress. Anxiety 31, 737–745.

Wijtenburg, S.A., Yang, S., Fischer, B.A., Rowland, L.M., 2015. In vivo assessment of neurotransmitters and modulators with magnetic resonance spectroscopy: application to schizophrenia. Neurosci. Biobehav. Rev. 51, 276–295.

Wolfe, J., Kimerling, R., Brown, P.J., 1996. Psychometric Review of the Life Stressor Checklist-revised. GEN. Instrumentation in Stress, Trauma, and Adaptation. Sidran Press.

Yuen, E.Y., Yan, Z., 2009. Dopamine D4 receptors regulate AMPA receptor trafficking and glutamatergic transmission in GABAergic interneurons of prefrontal cortex. J. Neurosci. 29, 550–562.

Yuen, E.Y., Liu, W., Karatsoreos, I.N., Ren, Y., Feng, J., McEwen, B.S., Yan, Z., 2011. Mechanisms for acute stress-induced enhancement of glutamatergic transmission and working memory. Mol. Psychiatry 16, 156–170.

Zwanzger, P., Zavorotnyy, M., Gencheva, E., Diemer, J., Kugel, H., Heindel, W., Ruland, T., Ohrmann, P., Arolt, V., Domschke, K., Pfleiderer, B., 2013. Acute shift in glutamate concentrations following experimentally induced panic with cholecystokinin tetrapeptide—a 3 T-MRS study in healthy subjects. Neuropsychopharmacology 38, 1648–1654.