NeuroImage: Clinical 14 (2017) 195–200

Contents lists available at ScienceDirect

NeuroImage: Clinical

journal homepage: www.elsevier.com/locate/ynicl

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

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ARTICLE INFO

*Article history:*
Received 21 November 2016
Received in revised form 23 December 2016
Accepted 2 January 2017
Available online 04 January 2017

*Keywords:*
Cortisol
Trier social stress test
$^{1}$H–MRS
Repeated scans

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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http://dx.doi.org/10.1016/j.nicl.2017.01.001

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 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).

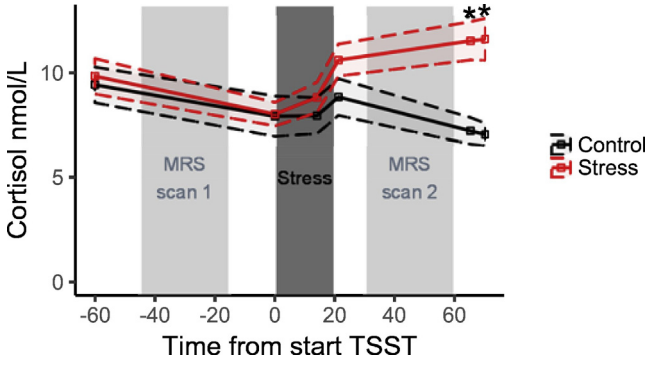


**Fig. 1.** Cortisol levels over time before and after exposure to the control condition ($N = 15$) or the stress condition ($N = 14$). The dotted lines represent the standard error. * = $p$-value < 0.01 (comparing the stress to the control condition in the posthoc test per time point).

### 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.

**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) |