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Is glutamate associated with fear extinction and cognitive behavior therapy outcome in OCD? A pilot study

M. Giménez[1,2] · M. Cano[1,2,3] · I. Martínez-Zalacaín[1,3] · E. Real[1,2] · P. Alonso[1,2,3] · C. Segalàs[1,2] · J. Munuera[4] · L. S. Kegeles[5,6] · J. J. Weinstein[5,6,7] · X. Xu[5,6] · J. M. Menchón[1,2,3] · N. Cardoner[8,9] · C. Soriano-Mas[1,2,10] · M. A. Fullana[2,9,11]

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Abstract

Cognitive behavioral therapy (CBT) including exposure and response prevention is a well-established treatment for obsessive–compulsive disorder (OCD) and is based on the principles of fear extinction. Fear extinction is linked to structural and functional variability in the ventromedial prefrontal cortex (vmPFC) and has been consistently associated with glutamate neurotransmission. The relationship between vmPFC glutamate and fear extinction and its effects on CBT outcome have not yet been explored in adults with OCD. We assessed glutamate levels in the vmPFC using 3T magnetic resonance spectroscopy, and fear extinction (learning and recall) using skin conductance responses during a 2-day experimental paradigm in OCD patients ($n = 17$) and in healthy controls (HC; $n = 13$). Obsessive–compulsive patients ($n = 12$) then received manualized CBT. Glutamate in the vmPFC was negatively associated with fear extinction recall and positively associated with CBT outcome (with higher glutamate levels predicting a better outcome) in OCD patients. Glutamate levels in the vmPFC in OCD patients were not significantly different from those in HC, and were not associated with OCD severity. Our results suggest that glutamate in the vmPFC is associated with fear extinction recall and CBT outcome in adult OCD patients.

Keywords Cognitive behavioral therapy (CBT) · Fear extinction · Glutamate · Obsessive–compulsive disorder (OCD) · Ventromedial prefrontal cortex (vmPFC)

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✉ M. A. Fullana
mafullana@clinic.cat

C. Soriano-Mas
csoriano@idibell.cat

1 Department of Psychiatry, Bellvitge University Hospital, Bellvitge Biomedical Research Institute-IDIBELL, Feixa Llarga s/n, Hospitalet de Llobregat, 08907 Barcelona, Spain

2 Carlos III Health Institute, Centro de Investigación Biomédica en Red de Salud Mental-CIBERSAM, Av. de Monforte de Lemos 5, 28029 Madrid, Spain

3 Department of Clinical Sciences, School of Medicine, University of Barcelona, Casanova 143, 08036 Barcelona, Spain

4 Diagnostic Imaging Department, Fundació de Recerca Hospital Sant Joan de Déu, Passeig Sant Joan de Déu, 2, Esplugues de Llobregat, 08950 Barcelona, Spain

5 Department of Psychiatry and Radiology, Columbia University, 622 W 168th St, New York 10032, USA

6 New York State Psychiatric Institute, 1051 Riverside Dr, New York 10032, USA

7 Department of Psychiatry, Stony Brook University, Stony Brook, 101 Nicolls Rd, Stony Brook, New York 11794, USA

8 Depression and Anxiety Program, Department of Mental Health, Parc Taulí Sabadell, Hospital Universitari, Parc Taulí 1, 08208 Sabadell, Spain

9 Department of Psychiatry and Legal Medicine, Universitat Autònoma de Barcelona, Av. de Can Domènech, 737, 08193 Cerdanyola Del Vallès Barcelona, Barcelona, Spain

10 Department of Psychobiology and Methodology of Health Sciences, Universitat Autònoma de Barcelona, Building B1, Ca n'Altayó, s/n, Bellaterra, 08193 Barcelona, Spain

11 Psychiatry Department, Hospital Clínic-Institute of Neurosciences, CIBERSAM, C/Rosselló 140, 08036 Barcelona, Spain

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Introduction

For almost a century, researchers have been interested in how organisms learn to fear and "not to fear" [1, 2]. This field has gained momentum during the past two decades due to its implications for our understanding of fear-related disorders and how to treat them [3–6]. Most research in this area has used Pavlovian fear (or threat) learning experiments, where a conditioned fear response is established (*fear conditioning*) through the repeated pairing of an initially neutral stimulus (the conditioned stimulus, CS) with a negative outcome (unconditioned stimulus, US). When the CS is later presented repeatedly without the US, the conditioned fear response decreases (*extinction learning* or within-session extinction). The memory of extinction learning can be probed later by presenting the CS again (*extinction recall* or between-session extinction).

Several lines of research have established that fear extinction processes are modulated by glutamate, a major excitatory neurotransmitter in the mammalian brain that is critical for learning and memory [7–11]. For example, rodent data suggest that neural plasticity associated with fear extinction requires membrane depolarization through activation of N-methyl-D-aspartate (NMDA) receptors [12], and NMDA agonists have been shown to facilitate fear extinction [13, 14]. More specifically, activation of NMDA receptors in the infralimbic cortex seems to mediate increased glutamate neurotransmission during consolidation of extinction learning [15], thereby facilitating successful extinction recall [16, 17]. In this regard, extinction learning and extinction recall in rodents may be altered by lesions [18–21] or direct electrical stimulation [22–24] of the infralimbic cortex. In healthy humans, structural and functional neuroimaging studies suggest that the ventromedial prefrontal cortex (vmPFC), the homologue of the rodent infralimbic cortex, is also important for both extinction learning [25] and recall [26, 27].

In clinical settings, cognitive behavior therapy (CBT) including exposure and response prevention is the first-line treatment for OCD [28–30] and is based upon the principles of fear extinction learning [12, 31]. Recent studies suggest that structural and functional changes in the vmPFC could predict CBT outcome in OCD. For example, thinner cortical thickness in the rostral anterior cingulate cortex (ACC)—a subregion of the vmPFC and decreased resting-state activity between the basolateral amygdala and the vmPFC predict a better CBT outcome in OCD adult patients [32, 33]. Moreover, glutamate-based therapies such as d-cycloserine have been shown to increase the success of CBT in humans [13, 34–38]; but see also [39, 40]. Thus, endogenous brain levels of glutamate may be associated with CBT outcome.

This possibility was recently explored in a randomized controlled trial of CBT in children/adolescents with OCD [41]. In this study, the authors found that lower pre-CBT levels of glutamate in the ventral posterior cingulate cortex (vPCC) were associated with better CBT outcome (i.e., greater symptom reduction). These authors had previously reported that lower pre-CBT levels of glutamate + glutamine (Glx) in the thalamus were also associated with better CBT outcome in children/adolescents with OCD [42]. Similarly, two studies in adults found decreased Glx and glutamate levels following CBT in the anterior middle cingulate cortex (aMCC) [43] and a sub-region of the vmPFC, the pregenual ACC [44].

To our knowledge, the association between brain glutamate and fear extinction has not yet been investigated in humans. Given the important role that fear extinction mechanisms may have in OCD [45], in the present pilot study, we evaluated whether glutamate levels in the vmPFC assessed using proton magnetic resonance spectroscopy (^{1}H-MRS) are associated with fear extinction learning and recall, as well as with CBT outcome, in adults with OCD. Moreover, we also included a healthy control (HC) group to control for potential baseline abnormalities in glutamate levels.

Methods

Overview

OCD patients and HC participated in a 2-day experimental protocol where glutamate levels in the vmPFC were measured using ^{1}H-MRS, and fear extinction (learning and recall) was assessed via skin conductance responses (SCR). Following the experimental protocol, OCD patients received a 20-week course of CBT. The study was performed in accordance with the requirements of the Institutional Review Board of University Hospital of Bellvitge and the ethical standards laid down in the 1964 Declaration of Helsinki and its later amendments. All participants gave written informed consent to participate.

Participants

We recruited patients from the OCD unit of Bellvitge University Hospital (Barcelona, Spain), and HCs (group matched by age and sex) from the local community through advertisements and word of mouth. Eligible patients were adults with a principal diagnosis of OCD (≥ 1 year) as established independently by two psychiatrists using the Structured Clinical Interview for DSM-IV Axis I Disorders (SCID-IV), clinician version [46].

OCD patients were eligible for CBT if they had a history of non-response (Yale-Brown Obsessive–Compulsive Scale [Y-BOCS] reduction < 35%) [47] or partial response (Y-BOCS reduction < 25%) to at least one 12 week trial with

a selective serotonin reuptake inhibitor (SSRI) following recommended guidelines [48], and had not previously received CBT. Pharmacological treatment remained at steady doses for at least 12 weeks before the study and was kept stable throughout the study. The ^{1}H-MRS and fear extinction data were collected 1–2 weeks before starting CBT.

The following exclusion criteria were applied to all participants: age < 18 or > 65; active substance use, abuse or dependence (except nicotine) during the previous 12 weeks; presence of personality disorders (as assessed by the Structured Clinical Interview for DSM-IV Axis II Disorders (SCID-II) [49], psychotic or bipolar disorders; mental retardation; presence or history of serious medical or neurological disorder (except a tic disorder); history of loss of consciousness for > 30 min; any contraindication for magnetic resonance imaging (MRI) scanning. Comorbid depressive and anxiety disorders were not considered to be exclusion criteria provided that OCD was the principal and most severe diagnosis.

The absence of mental disorders in the HC group was confirmed using the Spanish version of the Mini-International Neuropsychiatric Interview (MINI) [50], which was administered by a trained psychiatrist.

Fear conditioning and extinction procedure

We used the 2-day fear conditioning and extinction protocol developed by Milad et al. and adapted for use in the scanner [51]. Fear conditioning and extinction learning were assessed on day 1 and fear extinction recall on day 2 (24 h later). Briefly, the visual stimuli were photographs of rooms (two contexts) containing lamps of three different colors (CS). The US was a 100 ms (ms) electric shock delivered via electrodes attached to the dominant hand. During the *fear conditioning* phase, two CS (e.g., red and blue lights) were paired with the US 200 ms before the CS offset (CS+) 60% of times, and another CS (e.g., yellow light) was not (CS−). During the *extinction learning* phase, two of the three stimuli were presented, one CS + not followed by the US (extinguished CS + = CS + E) and the CS−. The other CS + was not presented during extinction learning (unextinguished CS + =CS + U). During the *extinction recall* phase, the CS + E, the CS + U, and the CS− were presented in the extinction context and no shocks were delivered. All phases of the experiment included 16 CS + (conditioning and recall, 8 CS + E and 8 CS + U; extinction learning, 16 CS + E) and 16 CS − trials. The CS + U and CS + E were counterbalanced across participants. The CS − trials were intermixed throughout all phases of the experiment among the CS + trials. For each trial, the room (context) was presented for 9 s (3 s alone and 6 s in combination with the CS + or CS −). The trials of the fear conditioning phase were presented in the conditioning context. The trials of the extinction learning and extinction recall phases were presented in the extinction context. The selection of the CS colors was counterbalanced across participants. The mean inter-trial interval was 15 s.

Skin conductance was recorded during the fear conditioning and extinction protocol as in previous studies [27, 51]. Skin conductance responses for each CS trial were calculated by subtracting the mean skin conductance level during the 2 s immediately before CS onset (during which the participants were presented with the context alone) from the highest skin conductance level recorded during the 6-sec CS duration, i.e., all reported SCR reflected changes in skin conductance above and beyond any changes produced by the context. Skin conductance data were normalized by square-root transformation, and when a SCR was negative, the absolute value was square-root transformed and the result was multiplied by − 1.

Neuroimaging data acquisition, preprocessing, and analyses

Neuroimaging data were acquired at 3T on a Siemens Magnetom Verio Syngo® MR B17 scanner equipped with 32-channel receive-only phased-array head coil (Siemens, Erlangen, Germany). For anatomical localization, a high-resolution structural MRI T1 sequence was obtained on day 1 (repetition time [TR] = 2100 ms, echo time [TE] = 2.67 ms, flip angle [FA] = 90º, field of view [FoV] = 256 × 256 pixel matrix, slice thickness = 1 mm), and the ^{1}H-MRS sequence was acquired on day 2. A single ^{1}H-MRS voxel ($20 \times 20 \times 20$ mm^3) was manually placed by an experienced MRI researcher (MC) in the vmPFC, centered mediolaterally on the midline and dorsoventrally at the most anterior point of the genu of the corpus callosum. To minimize the inclusion of cerebrospinal fluid, the posterior boundary of the voxel was located just rostral to the genu (see Fig. 1).

We acquired two separate ^{1}H-MRS sequences with two different TEs (short and long), using this same voxel and a standard point-resolved spectroscopy (PRESS) sequence with automatic shimming. For the first sequence, acquisition parameters were: TR = 2000 ms, TE = 30 ms, and 80 averages. For the second sequence, acquisition parameters were: TR = 2000 ms, TE = 135 ms, and 128 averages (FA = 90º in both acquisitions). Short- and long-echo times were compared to evaluate optimal parameters to detect glutamate levels, as previous authors have noted the challenges in accurate determination of glutamate concentrations in vivo [52–56].

Glutamate metabolite concentrations were measured using LCModel [57, 58] version 6.3-1H. The LCModel software uses a constrained non-linear least-squares analysis to estimate the metabolite concentrations, starting with solution spectra basis sets to provide concentration estimates without operator bias. LCModel also provides error estimates for the fit of each metabolite (% standard deviation

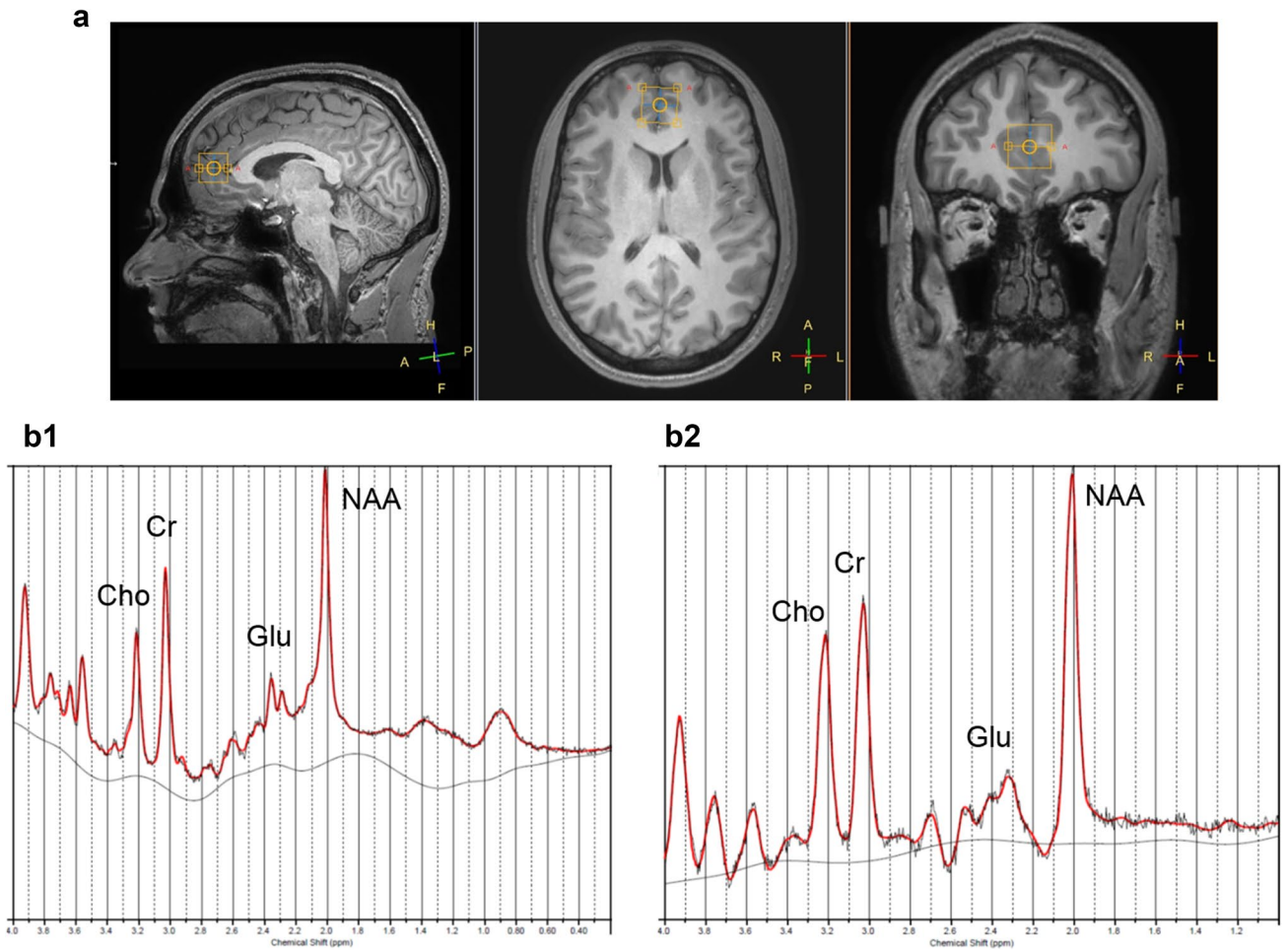

Fig. 1 **a** Single ^{1}H MSR-voxel localization [20×20×20 mm^{3}] in the vmPFC, and **b.1** typical spectral fit (red) of signal (black) from a TE30 ms acquisition and **b.2** typical spectral fit (red) of signal (black) from a TE135 ms acquisition. Institutional unit measure for metabolites. *Cho* choline, *Cr* creatine, *Glu* glutamate, *NAA* N-acetylaspartate, *ppm* parts per million

[SD] or Cramer–Rao lower bound [CRLB] variance). To decrease data variance, metabolite signals in the acquired spectrum were scaled to the signal of creatine (Cr) which appears to be stable within each tissue type; so, the relative values of glutamate vs. Cr were obtained (glutamate/Cr).

All spectra were visually inspected by three experts, who were blind to the study hypotheses. Cases were rejected if the fitted spectra showed: (1) poor fit for glutamate at its C4 proton multiplet resonance around 2.35 ppm (despite acceptable CRLB); (2) poor shim (full width at half maximum (FWHM) > 0.12 ppm for TE 135 ms and FWHM > 0.10 ppm for TE 30 ms); (3) low (< 8) signal-to-noise ratio [SNR]); or (4) poor distinction between major choline (Cho)/creatine (Cr) resonances at 3.2 ppm and 3.0 ppm, respectively. Thus, 2 OCD and 6 HC participants were excluded from analyses as both their TE 30 ms and TE 135 ms ^{1}H-MRS spectra failed to meet these criteria, giving a final sample of 30 participants (17 OCD and 13 HC). Moreover, based on these criteria, from this final sample of 30 subjects, 1 HC was excluded from the analyses of TE 135 ms spectra data, and 1 and 3 OCD patients were excluded from the analyses of the TE 30 ms and TE 135 ms spectra, respectively.

Cognitive behavior therapy and assessment of outcome

Cognitive behavior therapy focused on exposure and ritual prevention and was provided in group setting (6–8 attendees), except for 2 patients, who received individual CBT, by an experienced therapist who was blind to the study's hypotheses. Previous research suggests that group and individual CBT have a similar efficacy on OCD [59].

All patients received 20 weekly sessions lasting ~ 90–120 min each (45 min for the individual sessions) following Kozak and Foa [60] manual. The first two sessions focused on psychoeducation and introducing the behavioral model of OCD, and on developing an exposure hierarchy; sessions 3–18 consisted of gradual exposure (both imaginal

and in vivo) to items of the hierarchy, with instructions for strict response prevention from compulsions. The goal was for patients to stop their rituals as early as possible during therapy. Between sessions, participants were assigned 60 min homework per day, consisting of exposure to stimuli similar to those addressed in the sessions. The last two sessions focused on relapse prevention. Formal cognitive therapy techniques were not used, but dysfunctional cognitions were discussed within the context of exposure. Clinicians who were not involved in therapy assessed OCD symptoms and comorbid depressive symptoms before and after CBT using the Y-BOCS and the Hamilton Rating Scale for Depression (HAMD) [61].

We operationalized CBT outcome as the difference between Y-BOCS pre-CBT and Y-BOCS post-CBT. Of the 17 patients who initiated CBT, 5 did not finish the treatment, and therefore outcome data were available for 12 OCD patients.

Statistical analyses

In preliminary analyses, and for the whole sample, we compared the SCR during fear conditioning and extinction protocol using repeated-measures ANOVA for each phase (conditioning, extinction learning, and extinction recall), with CS type (CS + vs. CS− for conditioning and extinction learning and CS + E vs. CS + U for extinction recall) as within-subjects factors and group (OCD vs. HC) as between-group factor. Following Holt et al. [62], we divided conditioning into two blocks (early and late). The trials included in each phase are the same as those reported by Holt et al. [62].

Also, following Holt et al. [62], and for each individual, we calculated two fear extinction measures. The *extinction learning index* was calculated as the mean SCR for the last four CS + E trials minus that for the last four CS − trials during the extinction learning phase. Therefore, lower scores indicated enhanced extinction learning (i.e., less discrimination between CS + E and CS−). The *extinction recall index* was calculated as 100 − [(average SCR for the first four trials of extinction recall/largest SCR during fear conditioning) × 100]. Therefore, higher scores indicated enhanced extinction recall.

We analyzed the differences between groups (OCD vs. HC) in baseline characteristics, glutamate relative levels, and the two fear extinction indices (extinction learning and recall) using the Mann–Whitney *U* test or the Chi Square (χ^2) test, and the effect of CBT on OCD symptoms using the Wilcoxon signed-rank test for related samples. For this analysis, we also calculated Cliff's δ statistic [63] as an estimate of effect size. Note that a Cliff δ effect size of > 0.47 is considered "large" [64].We used Spearman's Rho (r_s) correlations to test the significance of the associations between glutamate relative levels and (i) OCD severity, (ii) fear extinction measures, and (iii) CBT outcome, as well as to calculate the correlation between both measurements of glutamate (TE 30 ms and TE 135 ms).

We initially set α at 0.05. In correlation analyses, a Bonferroni correction was applied to take into account the different correlations between glutamate and the behavioral and clinical measurements (i.e., glutamate × CBT outcome, glutamate x fear extinction learning, and glutamate × extinction recall; p 0.05/3 = 0.02). Finally, an evaluation of the effects of potential outlier values was also performed on our results.

Results

Preliminary analyses: fear conditioning and extinction procedure

Figure 2 displays the mean SCR on day 1 of the experiment. During conditioning, there were significant differences between the CS + (CS + E and CS + U) and CS− in the mean SCR within each group (both at early and late conditioning $p < 0.0005$), but the interaction group × CS type was not significant (early conditioning: $p = 0.142$; late conditioning: $p = 0.111$), thus indicating that both groups showed successful fear conditioning, but conditioning was not different between groups. During extinction learning, there were no significant differences between the CS + E and CS− in the mean SCR within each group ($p = 0.387$), and the interaction group × CS type was not significant either ($p = 0.635$), thus indicating that both groups showed successful extinction learning, and that extinction learning was not different between groups. During extinction recall, there were no significant differences between the CS + E and CS + U in the mean SCR within each group ($p = 0.740$), and the interaction group × CS type was not significant either ($p = 0.370$), thus indicating (similar to Milad et al. 2013) [45] that there were no significant differences between the two groups in the magnitude of the SCR during the recall phase.

Baseline characteristics and CBT outcome

The sociodemographic and clinical characteristics of the study participants are shown in Table 1. Glutamate levels in the vmPFC did not differ significantly between OCD and HC, for either TE 30 ms (OCD: mean, 1.136 ± SD, 0.223; HC 1.200 ± 0.178; U ($n_1 = 16$, $n_2 = 13$) 91.0, $p = 0.589$) or TE 135 ms (OCD: mean, 1.715 ± 0.433; HC 1.696 ± 0.267; U ($n_1 = 14$, $n_2 = 12$) 88.0, $p = 0.860$). Both TE 30 ms and TE 135 ms measures were highly correlated ($r_s = 0.715$, $p < 0.0005$). Moreover, glutamate levels were not significantly correlated with OCD severity (Y-BOCS pre CBT) (TE 30 ms: $r_s = 0.325$, $p = 0.219$; TE 135 ms, $r_s = -0.145$, $p = 0.621$).

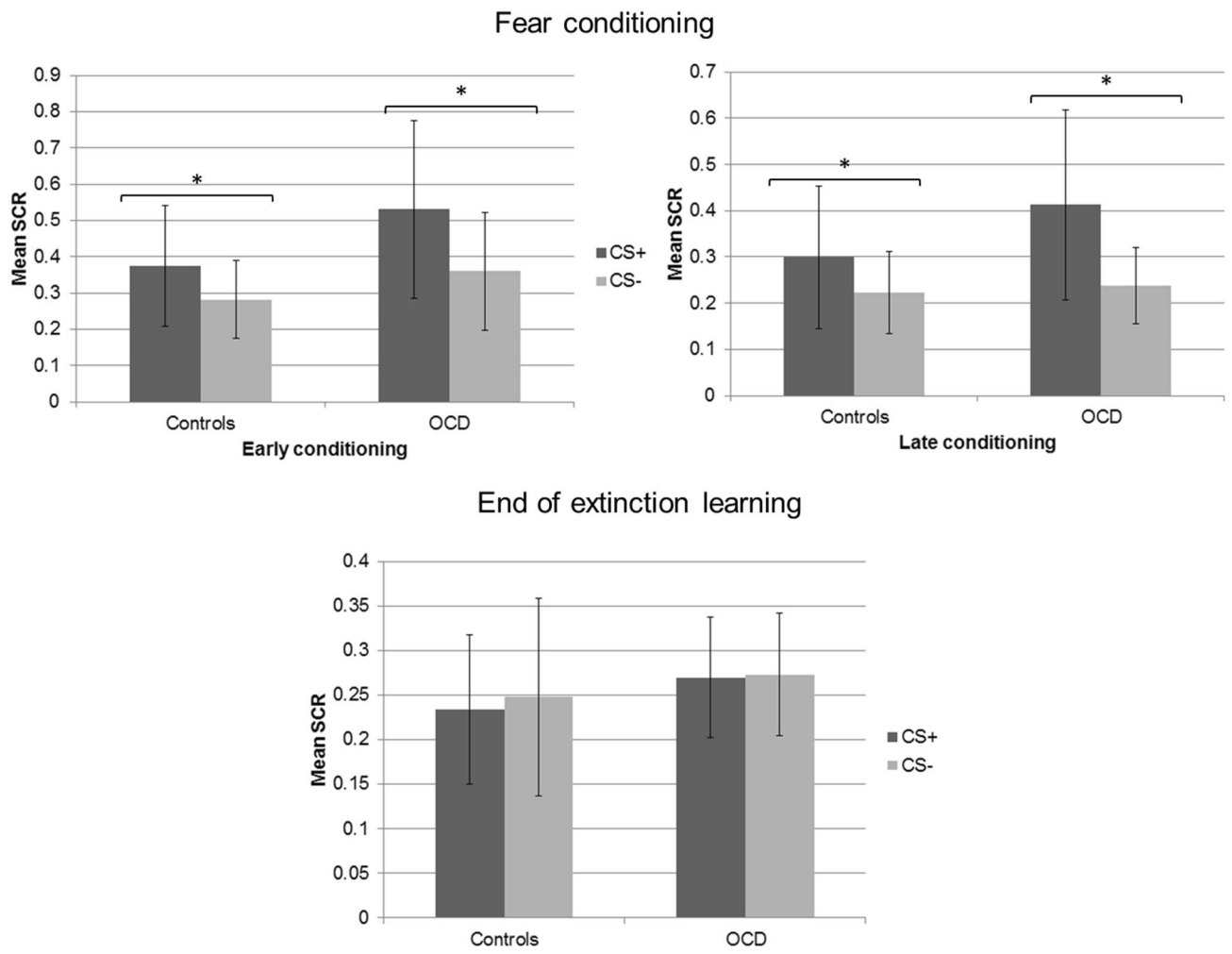

Fig. 2 Fear conditioning and extinction learning procedure: skin conductance responses (SCR) on day 1 of the experiment. Bars represent standard deviation (SD).*Significant within-group differences between CS− and CS+ during conditioning ($p < 0.0005$)

Cognitive behavior therapy was effective at reducing OCD symptoms (Y-BOCS pre CBT: 27.0 ± 5.1; Y-BOCS post CBT: 17.2 ± 7.0; Z ($n_1 = 12$, $n_2 = 12$) $= -3.063$, $p = 0.002$; effect size $\delta = 0.708$).

Glutamate levels and fear extinction indices

There were no differences between OCD and HC in our indices of extinction learning (OCD: -0.004 ± 0.061; HC -0.0135 ± 0.044; U ($n_1 = 17$, $n_2 = 13$) 106.0, $p = 0.869$) or extinction recall (OCD: 45.97 ± 18.76; HC 29.89 ± 45.51; U ($n_1 = 16$, $n_2 = 13$) 111.0, $p = 0.779$).

We did not observe any significant correlation between our extinction learning index and glutamate levels at TE 30 ms ($p > 0.3$) either considering the whole sample (OCD + HC) or the two groups separately. In contrast, for the whole sample, we observed a positive significant correlation between our extinction learning index and TE 135 ms glutamate levels ($r_s = 0.409$, $p = 0.038$), indicating that higher glutamate levels were associated with diminished extinction learning (see Supplementary Fig. 1). However, this correlation was no longer significant after Bonferroni correction. Within OCD patients, there was a trend toward a correlation for TE 135 ms ($r_s = 0.490$, $p = 0.075$). We also observed a significant negative correlation between our extinction recall index and TE 135 ms glutamate levels for the whole sample ($r_s = -0.483$, $p = 0.014$), i.e., higher glutamate levels were associated with diminished extinction recall, which remained significant after Bonferroni correction (see Supplementary Fig. 1). The difference in the correlation between groups approached significance ($p = 0.057$). In this sense, correlations of our extinction recall index with glutamate levels were not significant in the HC group for any TE ($p \geq 0.3$), while patients with OCD showed a significant negative correlation between our extinction recall index and TE

Table 1 Sociodemographic, clinical, and ^{1}H-MRS characteristics of study participants

Characteristic	OCD group $N=17$	HC group $N=13$	U or χ^2 tests	p value
Demographic data				
Sex (females/males)	10/7	8/5	0.023	1.000
Age (years) (mean ± SD)	40.2 ± 12.7	36.9 ± 12.8	128.5	0.457
Clinical data (mean ± SD)				
Age at OCD onset (years)	19.5 ± 10.4	–		
Y-BOCS score pre CBT	27.5 ± 4.4	–		
Y-BOCS score post CBT	17.2 ± 7.0	–		
Comorbidity (*n*, %)				
Affective disorders	5 (29.4)	–		
Anxiety disorders	2 (11.8)	–		
Pharmacological treatment[a] (*n*, %)	15 (88.2)			
Fluoxetine (20–80 mg/d)	4 (26.7)	–		
Fluvoxamine (100 mg/d)	1 (6.7)	–		
Sertraline (200 mg/d)	2 (13.3)	–		
Escitalopram (10–40 mg/d)	6 (40.0)	–		
Venlafaxine (75 mg/d)	1 (6.7)	–		
Paroxetine (40–60 mg/d)	2 (13.3)	–		
Glutamate values (mean ± SD)				
Relative value[b] TE30	1.136 ± 0.223	1.200 ± 0.178	91.0	0.589
Relative value[b] TE135	1.715 ± 0.433	1.696 ± 0.267	88.0	0.860
%SD[c] TE30 ms	6.412 ± 2.694	5.308 ± 1.601	78.0	0.161
%SD[c] TE135 ms	8.824 ± 3.302	7.615 ± 1.938	92.0	0.432

CBT cognitive behavioral therapy, *HC* healthy controls, *^{1}H-MRS* proton magnetic resonance imaging, *ms* milliseconds, *OCD* obsessive–compulsive disorder, *SD* standard deviation, *TE* echo time, *Y-BOCS* Yale-Brown Obsessive–Compulsive Scale

[a]At least 12 weeks of this dose was required before scanning and initiating CBT. Medication was kept stable throughout the study (except in two patients who were free of medication at the time of the ^{1}H-MRS)

[b]Metabolite signals in the acquired spectrum were scaled to the signal of creatine (Cr)

[c]Standard error estimates (Cramer–Rao lower bounds of the fit, expressed as %SD by the LCModel software)

135 ms glutamate levels ($r_s = -0.747$, $p = 0.003$, significant after Bonferroni correction). This result is depicted in Fig. 3a.

Glutamate levels and CBT outcome

There was a significant positive correlation between glutamate levels before starting CBT (baseline) and CBT outcome (Y-BOCS pre-CBT-Y-BOCS post-CBT) with both TE 30 ms ($r_s = 0.685$, $p = 0.020$) and TE 135 ms ($r_s = 0.748$, $p = 0.013$) measurements, indicating that higher levels of glutamate in the vmPFC were associated with a better CBT outcome (reduced OCD severity) (Fig. 3). Both correlations remained significant after Bonferroni correction.

No outlier subjects were detected (values > 1.5 of the interquartile range) in the variables evaluated. This was confirmed by the visual inspection of the plots (Fig. 3).

Discussion

The results of this pilot study suggest that glutamate in the vmPFC is associated with both fear extinction recall and CBT outcome in OCD. Specifically, we observed that glutamate was *negatively* associated with fear extinction recall and *positively* associated with CBT outcome.

To our knowledge, this is the first demonstration of an association between glutamate in the vmPFC and fear extinction recall (as assessed with the extinction retention index) in OCD patients. As noted in the introduction, previous research in rodents has established that both glutamatergic neurotransmission and the infralimbic cortex (the homologue of the human vmPFC) are important for extinction recall. Our results expand these animal data to OCD patients. We found that higher glutamate levels in the vmPFC in OCD patients were associated with diminished extinction recall, even though there was no

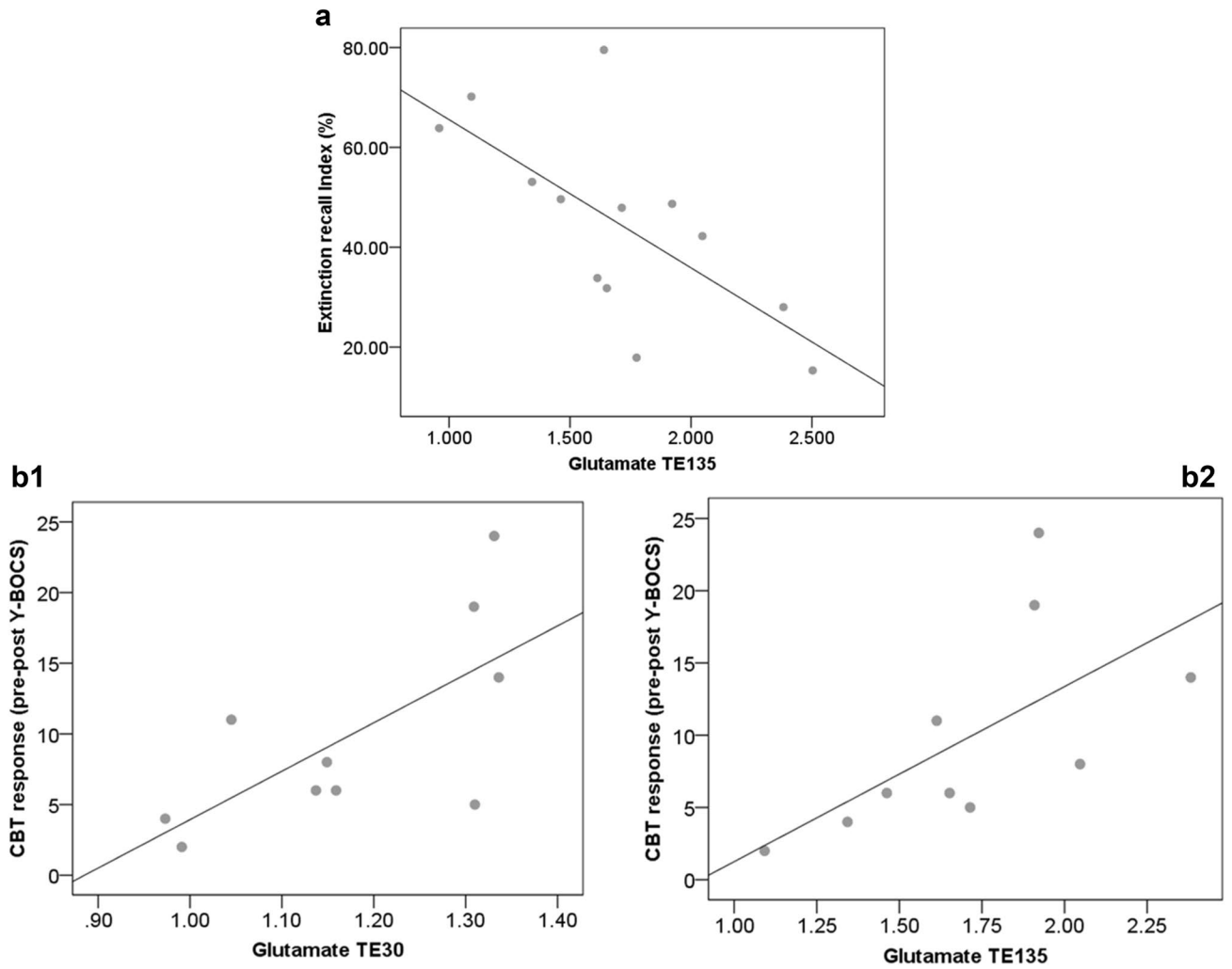

Fig. 3 Correlations between glutamate measures, extinction recall index, and CBT

difference in either glutamate levels or extinction recall between patients and controls. While previous studies in adults have not observed differences in Glx levels between OCD and HC in the orbitofrontal [65] and mPFC [66], Glx has been found to be reduced in pediatric OCD samples [67]. Thus, prefrontal glutamate changes in OCD may interact with brain development or disorder history, shifting from reduced levels during childhood to "normal" levels in adulthood, which could be, however, associated with impaired extinction recall. Of note, a previous study in healthy adults found that greater vmPFC activity—as measured with functional magnetic resonance imaging (fMRI)—correlated with better extinction recall [51], which seems in contrast to the current findings. Future studies may combine both magnetic resonance techniques—spectroscopy and fMRI—in the same participants to elucidate this apparent contradiction.

In contrast, we were not able to find a clear association between fear extinction learning and glutamate in OCD (we only found a main effect for the whole sample, in line with the extinction recall–glutamate relationship, the more the glutamate levels, the less the extinction learning). We focused on glutamate in the vmPFC because previous task-based functional studies in humans have showed that the vmPFC is activated during fear extinction processes [25]; however, this study highlighted that vmPFC activation was primarily linked to extinction recall processes [25]. Some lesion studies also support the idea that the vmPFC is more critical for extinction recall, than for extinction learning [18, 21]. Therefore, it is possible that extinction learning could be mostly driven by glutamate levels in other brain regions.

Glutamate levels in the vmPFC were, however, positively associated with CBT outcomes in our OCD sample. Although our results have to be interpreted with caution

given the pilot nature of our study, they seem to confirm (and expand to an adult population) the role of glutamate as a potential CBT treatment biomarker in OCD, as recently suggested by O'Neill et al. [41]. In their pediatric sample, O'Neill et al. found a *negative* association between glutamate levels in the vPCC and CBT outcome, in contrast to the *positive* association we observed in the vmPFC in this study. There are at least two possible explanations for these differences. First, as mentioned above, brain development or disorder history may modulate the effect of glutamate on OCD. Second, here we focused on the vmPFC because of its putative role in fear extinction, and we did not assess the vPCC.

Our findings of increased glutamate associated with *reduced* extinction recall and better CBT outcome may seem contradictory, given that *increased* extinction recall has been found in at least one study to be associated with better CBT outcome in social anxiety disorder [68]. However, it is possible that individuals with increased vmPFC glutamate levels may show reduced extinction recall, and, at the same time, these individuals may also benefit more from CBT, which is aimed at improving such "deficient" extinction recall (see [33]). Moreover, although both extinction learning and recall could be proxies for processes occurring during CBT, it is unclear which is more strongly associated with CBT. Despite previous evidence indicating that CBT is associated with fear extinction recall [68], recent reports also establish a link between CBT and extinction learning [69, 70]. In any case, extinction learning and recall seem to be independent processes, which may be differently associated with CBT outcome [25, 51, 71]. In this context, it is important to note that Milad et al. [45] found diminished extinction recall in patients with OCD, but not impaired extinction learning. Nevertheless, in our study, we did not observe such dissociation between extinction learning and recall, and this may be partially explained by methodological reasons, such as sample size (21 vs. 13 patients with OCD in our study) or the differences in symptom severity between samples (mean Y-BOCS scores of 22.6 vs. 27.5 in our study).

Finally, it should be also mentioned that, contrary to previous reports [72], we did not find an association between glutamate levels and the severity of OCD symptoms. However, in that manuscript Yücel and colleagues' reported [72] glutamate levels from a more dorsal anterior cingulate cortex cluster in a sample of women with moderate symptom severity (mean Y-BOCS score of 19). In contrast, our sample included men and women with moderate-to-severe symptoms (mean Y-BOCS score of 28). Thus, the differences between the results of these studies may be partly explained by differences in symptom severity in addition to a different location of the ^{1}H-MRS cluster.

This study is not without limitations. First, although main results were relatively robust (significant after Bonferroni correction), they should be seen as preliminary and replicated in wider samples to ensure generalizability. Of note, however, at least two previous studies have also found significant associations between brain metabolite levels (Glx, among others) and CBT in smaller samples (<i.e., 10 subjects or less per group) [42, 43]. Second, almost all OCD patients in our study were taking medication, and some research suggests that SSRIs may affect glutamate levels [73] or fear extinction processes [74], although results are inconclusive (see, e.g., [75, 76]). In any case, we included patients who remained symptomatic after drug treatment, as most patients with OCD are unlikely to receive CBT as the first treatment option [77]. Therefore, our administration of CBT is similar to real clinical practice [78]. Third, glutamate measurements are heavily coupled to glutamine estimates, and various reports coincide in suggesting that short TEs (i.e., TE 30 ms) should be reported in combination with strongly coupled measurements [79–81]. However, we analyzed short and long TEs, and our most robust results were obtained with TE 135 ms. Anyhow, our TE30 ms and TE135 ms measurements were positively correlated, and previous studies have found no conclusive differences between short and long TEs [82]. Finally, the fact that MRS data were acquired during the second MRI session may partially account for the lack of association between glutamate and fear learning in OCD.

To sum up, we have reported an association between glutamate levels in the vmPFC and both fear extinction recall and CBT outcome in adult OCD. We found a significant (and opposite) relationship between these measurements and glutamate levels in the vmPFC. While our methodological approach do not allow us to directly compare our results with those of previous studies and our results should be seen as preliminary, they can be interpreted against the background of previous findings using other neuroimaging techniques showing a clear relationship between vmPFC structure and function and extinction recall and CBT response in OCD [26, 27, 33]. Further research should try to integrate all these different measurements from experimental and clinical settings and, from a multimodal neuroimaging perspective, aim to provide a thorough description of the role of the vmPFC and glutamate neurotransmission in fear extinction and CBT.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical standards The study has been approved by the Institutional Review Board of University Hospital of Bellvitge and it has been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki ad its later amendments. All participants gave written informed consent to participate.

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