INTRODUCTION

Eye Movement Desensitization and Reprocessing (EMDR) is a psychotherapy that has been found to effectively resolve the effects of traumatic experiences and following numerous randomized clinical trials in patients with post-traumatic stress disorder (PTSD) it has been recognized as a first-line treatment for PTSD.
PTSD is characterized by dysfunction and structural alteration of several discrete brain regions. Neurobiological investigations of PTSD have shown that it may be characterized by lower density in limbic and paralimbic cortices, with changes in gray and white matter volume and concentration (GMV and GMC, respectively) in hippocampus, parahippocampal gyrus and cingulum. However, possibly due to the high heterogeneity of traumatic events causing PTSD and of patients symptoms (i.e. hyperarousal vs dissociation) as well as of cohort sizes a surprisingly large variance across studies has been reported.

Most Magnetic Resonance Imaging (MRI) studies on PTSD have measured volumetric changes in discrete brain regions or small brain structures. Karl et al. in a meta-analysis of structural brain MRI in PTSD concluded that the disorder is associated with abnormalities in multiple frontal-limbic system structures, notably in hippocampi, amygdala, and anterior cingulate cortex. Similarly, a recent meta-analysis by Woon et al. showed a lower grey matter density in limbic and paralimbic cortices to be associated with PTSD diagnosis.

Studies investigating the effect of Cognitive Behavioural therapy (CBT) on hippocampal volume in PTSD patients have reported conflicting results. Recently functional studies have reported EMDR-related neurobiological changes and our group has investigated the structural changes after EMDR therapy.

The aim of the present study was to extend such investigation beyond the regional assessment computing in PTSD patients and healthy controls a voxel-wise analysis on the hippocampal volume reduction in individuals with PTSD.

Furthermore, investigating the changes in GMC in patients with and without PTSD, Zhang et al. found those with PTSD showing significantly decreased GMC in left anterior hippocampus and left parahippocampal gyrus and Nardo et al. showed a lower grey matter density in limbic and paralimbic cortices to be associated with PTSD diagnosis.

A comprehensive psychiatric evaluation was conducted at baseline. Psychiatric diagnoses based on DSM-IV and on the Structured Clinical Interview for DSM-IV (SCID) were determined by a consensus of two psychiatrists not otherwise involved in the study. Healthy controls were assessed with the SCID. Patients were evaluated with the SCID-Non-Patient version. Patients were evaluated with the SCID for DSM-IV Axis I (SCID-I/P) and Axis II (SCID-II/P) disorders in order to determine a single diagnosis of PTSD, and were assessed with the Clinician Administered PTSD Scale (CAPS) Italian Version, which is known to be a reliable measure of PTSD severity with subcomponents for the individual symptom clusters. We administered the Davidson Trauma Scale (DTS), a dimensional measure of PTSD with 17 items (with scores ranging from 0-136) for PTSD severity. An evaluation for the presence of overlapping symptoms between PTSD, Major Depressive Disorder, and state of anxiety was also performed respectively with the Hamilton Depression Rating Scale (HAM-D) and the Hamilton Anxiety Rating Scale (HAM-A). At post-treatment, the CAPS was administered to patients again.

**METHOD**

**Participants**

Thirty-eight participants were studied: 19 drug-naive patients with PTSD (10 men and 9 female) and 19 age matched healthy controls (15 men and 4 women). The patient group was recruited at the Center for the Diagnosis and Treatment of Post-Traumatic Stress Disorder, Department of Psychiatry, University of Siena, between September 2010 to May 2012 and largely overlapped the cohort recruited for a previous study. Patient inclusion criteria were: age between 18 and 65 years and the drug-naive status. Exclusion criteria were: history of current and/or lifetime comorbid psychiatric diagnoses as determined by the SCID; previous or current use of any psychotropic medications; history of head trauma; presence of neurological, endocrine, or degenerative disorders. Healthy controls were recruited at the hospital “Le Scotte” in Siena, Italy, and matched for age, education, handedness, weight and height. Exclusion criteria for controls were: a history of meningitis, traumatic brain injury, presence of neurological, en-
Neuroradiological acquisition

MRI examinations of all participants were performed at a 1.5 Tesla Philips Intera scanner (Philips Medical Systems, Best, The Netherlands). Morphovolumetric analysis were run onto T1-weighted Fast Field Echo (FFE) 1-mm thick images of the entire brain (TR/TE=30.00/4.6 ms, flip angle=30.00, FOV=250 mm, matrix 256x256, slice number=75) followed by the axial plane parallel to the anterior and posterior commissures. Neuroradiological examination also included FFE 1-mm thick coronal images (TR=30.00/4.6 ms, flip angle=30.00, FOV=250 mm, matrix 512x512, slice number=75), T2-weighted Turbo Fluid Attenuated Inversion Recovery (FLAIR) 3-mm-thick axial images (TR/TE=9000/110ms, IR delay=2500ms, FOV=230mm, matrix 512x512, slice number=40).

Optimized voxel-based morphometry

An optimized voxel-based morphometry (VBM) protocol was performed (i) at baseline (Time 1), to determine any abnormality of grey matter concentration (GMC) and volume (GMV) in patients compared to healthy participants, and (ii) post-EMDR (Time 2) to evaluate longitudinal changes in regional brain volumes of patients. For image pre-processing we used the freely available SPM8 software package (Statistical Parametric Mapping software: http://www.fil.ion.ucl.ac.uk/spm/) implemented in Matlab 7.11 (Math-Works Inc., Sherborn, MA). Pre-processing included “unified” segmentation and spatial registration as implemented in the tool “new segment” followed by deformetric registration22 of the grey and white matter probability maps derived from the previous step and affine-only registration to the standardized Montreal Neurological Institute - MNI space (ICBM 152, Montreal Neurological Institute standard T1-weighted template). Hidden Markov Random Field model was applied in all segmentation processes in order to remove isolated voxels. The customized prior images and T1-weighted template were smoothed using an 8 mm Full-Width at Half-Maximum Isotropic Gaussian Kernel (FWHM IGK). Modulated gray matter images were smoothed using an 8-mm FWHM IGK for gray matter volume analysis, unmodulated gray matter images were smoothed using a 12-mm FWHM IGK for GMC analysis. Differently from cross-sectional analysis, where data images can be processed independently for each participant, by deformetric effect evaluation we adopted special analysis parameters23,25. Each participant image was registered to mean baseline image and spatial normalization process was applied only for the baseline image and then applied to all images.

Statistical analysis

Cross-sectional comparisons between PTSD and control groups at baseline

One-way Analysis of CoVariance (ANCOVA) models as implemented in SPM8 were applied. We compared GMV changes across groups covarying for age, gender and Total Intracranial Volume (sum of gray and white matter tissues maps, TIV). GMC group differences were assessed using age and gender as covariates. Multiple comparison corrections were performed using Monte Carlo simulation (corrected p<0.05), taking into account both the individual voxel probability threshold and voxel cluster size in order to establish the probability of false-positive detection (cluster connection radius 4 mm, individual voxel threshold p<0.01, iterations=1000, FWHM=8 mm, inclusive masks obtained by averaging participants grey matter tissue maps). All results were reported using MNI coordinate system. Anatomical localization of significant clusters was performed using ANATOMY toolbox for SPM8 (http://www.fz-juelich.de/inm/inm-DE/Forschung/docs/SPMANatomyToolbox).

RESULTS

Patients and controls did not statistically differ for demographic data, as reported in Table 1. Patients’ diagnoses of PTSD at baseline (T1) were confirmed by clinical evaluation and by the fulfillment of all the criteria at CAPS. All patients had experienced a one-time adult trauma: natural disaster (n=3), sudden death of a family member (n=5), car accident (n=2), assault/robbery (n=6), and terrorist attack (n=4). One patient dropped out because of a depressive episode onset and consequently we removed a matched healthy control participant.

Baseline comparisons between patients and controls: Grey Matter Volume

The GMV comparison between patients and healthy participants at baseline showed significant differences (F 1.35=3.674; p=.008; η²=.298). Analyses revealed a region of significantly decreased GMV in patients’ left parahippocam-
pa region, supplementary motor area, lingual gyrus, and both left and right superior frontal gyrus. Patients with PTSD also showed a significant increase in GMV corresponding to right angular gyrus, inferior parietal lobule and left inferior temporal gyrus. MNI coordinates of each significant cluster, F-values and clusters dimension are reported in Table 2.

### Baseline comparisons between patients and controls: Grey Matter Concentration

The GMC comparison between patients and healthy participants at baseline did not show any significant differences (F1,35=0.984; p=.332).

### Longitudinal comparisons for patients’ clinical PTSD symptom scales pre and post EMDR

During the baseline assessment, patients showed a moderate to severe PTSD symptom severity, as highlighted by the DTS values: DTS total score was 99 +/- 9 with mean scores for each subscale of Intrusion 32 +/- 9, Avoidance 40 +/- 14 and Hypervigilance 27 +/- 9. At pre-treatment, the mean CAPS total score was 75.8 (+/- 21.8), with mean score for re-experiencing subscale of 17.0 +/- 8, avoidance 20.5 +/- 9.0; and hyperarousal 18.5 +/- 9.8. After 12 sessions of EMDR (Time 2), there was a significant pre-post decrease on the mean CAPS total score (19.3 +/- 15.5) (t (35)=2.132, p<.004) and hyperarousal subscale (4.1 +/- 9.8; p<.001) (t (35)=1.347, p<.008), and a non-significant trend to decrease on the re-experiencing (6.8 +/- 8.0) and avoidance (9.8 +/- 9.0) subscales. All 19 patients completed EMDR therapy and reported improvements in their PTSD symptoms, with 16 patients no longer satisfying necessary criteria for PTSD diagnosis.

### Longitudinal comparisons between patients and controls: Grey Matter Volume

Group-time interactions for GMV maps were significant (F (1,35)=4.324; p=.006; η²=.398), indicating a larger increase in GMV in patients as compared to healthy controls, specifically for left parahippocampal gyrus (F (1, 35)=11.237; p=.001, MNI x=-24, y=-21, z=-29; voxels=246), where patients had showed a significantly smaller GMV compared to controls before the EMDR treatment (Figure 1). Additionally, in comparison to healthy controls, a cluster of decreased GMV was found in patients’ left thalamus region after EMDR treatment (F (1, 35)=9.432; p=.002, MNI x=-9, y=-24, z=6; voxels=168) (Figure 2). No differences between first and second MRI acquisition were highlighted for healthy control participants (F 1,35=0.0146; p=.389).

### Longitudinal comparisons between patients and controls: Grey Matter Concentration

The ANCOVA comparing baseline and post-EMDR GMC and VCBT did not show any significant differences (F1,35=0.989; p=.421).

### DISCUSSION

In this study brain MRI measurements with Optimized Voxel-Based Morphometry was used to investigate the neurobiological effects of EMDR treatment in drug-naïve PTSD without comorbidity. Consistent with other volumetric findings26,27, when we compared patients with PTSD to healthy...
controls at baseline, we found significantly smaller GMV in the patients’ parahippocampal, parietal and frontal regions, and significantly larger GMV in temporal and parietal areas (Table 2) all regions involved in processing and storing mechanism of traumatic events\(^27\). Furthermore, after treatment completion comparisons with baseline showed in patients a significant increase in GMV in left parahippocampal gyrus and a significant GMV decrease in left thalamus. The implementation of VBM has allowed to extend the structural analysis to the entire brain overcoming the limitation of our previous investigations restricting the assessment of the effect of EMDR to the hippocampal region. Structural evaluation provides understanding of a disorder’s neurobiological substrate and allows to anatomically identify and measure changes which have clinical implications. Although to date we are still far from matching symptoms and single alterations, several works investigating PTSD suggested that many symptoms and/or psychopathological characterizations appear to be closely related to some specific neurobiological alterations. In the present study hippocampus, the main site for short-term memory processing, was found at baseline significantly smaller than in healthy controls and its volume increased following successful EMDR therapy. Hippocampus is involved in encoding, consolidating and retrieving declarative memories\(^28,29\) and receives extensive inputs from several regions of the neocortex\(^30,31\). Hippocampal dysfunction has been claimed to play a key role in the memory disturbances considered to be the core component in PTSD\(^5-7\) and it is known by long that PTSD is associated with abnormalities in activity and volume of the hippocampus\(^32\), as is it true in the symptomatic phase for our patients. It has been speculated that in PTSD emotional information is retained in amygdala and hippocampus and this pathological condition might be related to hippocampal volume reduction, possibly due to the effect of chronic release of cortisol, affecting specifically this brain region. Moreover, a failure in the func-

**Figure 1.** Significant increased GMV post-EMDR in patients’ left parahippocampal gyrus. Panel A shows coronal and axial views of increased grey matter volume in left hippocampus area of patients with PTSD (p<0.001 uncorrected; p<0.05 using MonteCarlo correction for multiple comparisons).

**Figure 2.** Significant decreased GMV post-EMDR in patients’ bilateral thalamus regions. Panel A shows coronal and axial views of decreased grey matter volume in left thalamus of PTSD patients.
Morphovolumetric changes after EMDR treatment in drug-naïve PTSD patients

Riv Psichiatr 2017; 52(1): 24-31

...A cluster of decreased GMV in patients' left thalamus region after EMDR treatment. Decreases in thalamic volume of thalamic hyperactivation and hyper-function were correlated with PTSD re-experiencing symptoms. Thalamus was also shown by fMRI to be hyperactivated in stress-induced analgesia, non-conscious fear and alexithymia. A possible explanation for our findings is that post-EMDR decrease in re-experiencing symptoms, also if a large but not significant trend, has resettled the normal functioning of the thalamus and somehow diminishes its hyperactivation resulting in parallel changes at a structural level. On the other hand it has been reported that emotional processing is linked to anterior and posterior cingulate cortex co-activation mediated by the thalamus. The clinical efficacy of EMDR reducing the emotional valence of the event might have also contributed to a decrease of thalamic involvement in trauma re-living and hence to a volume decrease as compared to the symptomatic phase. The structural changes found after EMDR treatment as compared to the symptomatic phase paralleled the significant decrease in clinical (CAPS) ones, mainly due to the hyperarousal sub-scale but also determined by the sub-threshold decreases in the re-experiencing and avoidance sub-scales. The latter results showing a mere trend to significant changes could be due to the small sample size increasing the likelihood not only of type I (false positive) but also type II (false negative) errors. EMDR is widely recognized as an efficacious treatment for PTSD with high rates of elimination of the diagnosis of PTSD. Beside, its neurobiological correlates have been recently investigated and reported. According to our results the clinical effectiveness of EMDR may lay in the restoration of hippocampus structure. Following EMDR treatment, we observed in this region an increase in GMV speaking in favour of a parallel recovering in hippocampal functions and cortical networking, with successful processing of the traumatic memory and resolution of current triggers. Also if its mechanism of action is beyond the purposes of this paper our results suggest that the EMDR leads to a better hippocampal functioning favoring neuronal integration between subcortical areas and cerebral cortex. This is in agreement with previous investigations in which the memory retention of the traumatic event was shown to move from emotional limbic subcortical regions including parahippocampal gyrus to an explicit cortical ones and elaborated at higher cognitive levels. On the other hand working memory processed by hippocampus has been hypothesized to be a determinant of EMDR effectiveness. The reduced vividness of emotional memory during bilateral stimulation might be due to limited working memory resources and this would be in accordance with the reduced hippocampal volume found in PTSD patients at baseline. In this respect we provided preliminary evidence that effective treatment may be reflected in anatomical and functional changes in some critical areas altered by PTSD and this has in perspective a great utility, producing more objective measurement than the self-report measures typically used in treatment outcome studies. Such insights could be used to select treatments which could target specific neurobiological alterations, with the goal of achieving resolution of biological damage and, subsequently, the disorder.

LIMITATIONS AND RECOMMENDATIONS

One limitation of this study is the small sample size possibly overestimating the number of foci showing significant differences. On the other hand the relative high costs of the methodology, makes the recruitment of an inadequate number of subjects to be investigated a common limitation in neuroimaging studies. For this reason in our study as in other ones in the past patients recruitment and characterization suffer of the presence of different trauma types and of discrepancies about the number of previous traumas, both issues potentially biasing the results. We also acknowledge that the recruitment of PTSD patients without comorbidity and of non-traumatized control subjects might render the results of the present investigation not directly comparable to other studies in the same field. However, the with-in subject analysis strengthened, along with the objective decrease of PTSD clinical scores, the reliability of the pre- to post-therapy changes and in the most of the control subjects mix lifetime traumas, even if not causing symptoms have certainly happened. Furthermore, the absence of follow-up to evaluate the maintenance of symptomatic improvement and the volumetric changes does not allow to draw conclusion on the long-term effectiveness of EMDR therapy. Future research might benefit of optimized voxel based morphometry and by the use of diffusion weighted images acquisition aimed at white matter fiber tracts changes detection, to examine the possible impact of psychotherapies on brain structural connectivity.

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Riv Psychiatr 2017; 52(1): 24-31

30
Morphovolumetric changes after EMDR treatment in drug-naïve PTSD patients