

# Emotional reactivity in chronic schizophrenia: structural and functional brain correlates and the influence of adverse childhood experiences

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**Background.** Despite behavioural signs of flattened affect, patients affected by schizophrenia show enhanced sensitivity to negative stimuli. The current literature concerning neural circuitry for emotions supports dysregulations of cortico-limbic networks, but gives contrasting results. Adverse childhood experiences (ACEs) could persistently influence emotional regulation and neural correlates of response to emotional stimuli in healthy humans. This study evaluated the effect of ACEs and chronic undifferentiated schizophrenia on neural responses to emotional stimuli (negative facial expression).

**Method.** Brain blood-oxygen-level-dependent functional magnetic resonance imaging neural responses to a face-matching paradigm, and regional grey matter (GM) volumes were studied at 3.0 T in the amygdala, hippocampus, anterior cingulate cortex (ACC) and prefrontal cortex (PFC). The severity of ACEs was assessed. Participants included 20 consecutively admitted in-patients affected by chronic undifferentiated schizophrenia, and 20 unrelated healthy volunteers from the general population.

**Results.** Patients reported higher ACEs than controls. Worse ACEs proportionally led to decreasing responses in the amygdala and hippocampus, and to increasing responses in the PFC and ACC in all participants. Patients showed higher activations in the amygdala and hippocampus, and lower activations in the PFC and ACC. Higher ACEs were associated with higher GM volumes in the PFC and ACC, and schizophrenia was associated with GM reduction in all studied regions.

**Conclusions.** Structural and functional brain correlates of emotional reactivity are influenced by both current chronic undifferentiated schizophrenia and the severity of past ACEs.

Received 17 November 2009; Revised 16 April 2010; Accepted 29 April 2010; First published online 9 June 2010

**Key words:** Adverse childhood experiences, emotion, grey matter, schizophrenia.

## Introduction

Flattened affect is a core symptom of schizophrenia, but the restriction in range and intensity of emotional expression does not seem to reflect a diminished subjective experience of emotion (Kring *et al.* 1993). One study showed that in their daily life patients experienced negative emotions with the same intensity and variability of control subjects, but with a reduction of positive emotional experiences which correlated with a reduction in positive environmental stimulation

(Myin-Germeys *et al.* 2000). In experimental conditions patients showed a lower ability to amplify, but not to suppress, positive emotions (Henry *et al.* 2007), and at an automatic processing level they showed a higher sensitivity for negative affect (Hoschel & Irle, 2001) and a lower sensitivity to positive affect (Suslow *et al.* 2003). Patients with schizophrenia appear to be accurate perceivers of others' emotional attitudes (Tompson *et al.* 1995; Sczufca *et al.* 2001), and hardly tolerate highly expressed emotions and criticism from those closest to them and most influential in their life (influential others) (Barrelet *et al.* 1990; Cutting *et al.* 2006).

Studies of brain imaging correlates of human emotional processing, joined with structural data from human and animal research, have allowed experts in

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the field to define a network of structures which provide a neural circuitry for emotions. The amygdala is a core component of the network and several cortico-limbic structures interact to identify the emotional significance of the stimuli and to generate and regulate affective states (Phillips *et al.* 2003*a, b*). This network can be studied with functional magnetic resonance imaging (fMRI) paradigms, which allowed the definition of the effective connectivity of the amygdala and its activation in response to negative facial expressions (Stein *et al.* 2007). Functional impairments of this network could lead to abnormal emotional processing and misinterpretations of interpersonal communication, which are believed to be key mechanisms in the development of psychosis (Phillips *et al.* 2003*b*).

Converging evidence suggests that the structural integrity of amygdalo-cortical circuits could be disrupted in schizophrenia, and it is also hypothesized that abnormal changes within these limbic circuitries could play a major role in the pathogenesis of the disorder (Benes, 2010). A quantitative review of studies investigating the relationship between symptom dimensions and blood-oxygen-level-dependent (BOLD) fMRI correlates of neural activity linked abnormal amygdala activity during emotional processing with greater psychotic symptoms and with emotional blunting (Li *et al.* 2009; Goghari *et al.* 2010).

Contrasting observations make it, however, difficult to draw conclusions on the topic. In particular, paranoid patients showed reduced amygdala responses to fearful faces in comparison with healthy controls and non-paranoid patients (Williams *et al.* 2004, 2007), and higher responses to neutral and positive stimuli (Surguladze *et al.* 2006). Other studies associated schizophrenia with a failure to activate the amygdala in response to fearful facial expressions (Phillips *et al.* 1999; Fakra *et al.* 2008; Rasetti *et al.* 2009), aversive scenes (Taylor *et al.* 2002), sad mood induction (Schneider *et al.* 1998) and mixed facial emotion discrimination (Gur *et al.* 2002). However, opposite observations of increased amygdala response to happy faces (Kosaka *et al.* 2002), to the passive viewing of fearful stimuli (Holt *et al.* 2006), to emotional words (Sanjuan *et al.* 2007), to emotional pictures (Taylor *et al.* 2005), and to implicit and explicit emotional processing (Blasi *et al.* 2009) have been reported too, and have been shown to normalize after successful antipsychotic treatment (Blasi *et al.* 2009). Furthermore, one study reported that patients showed neural responses similar to controls when performance was not considered, but they showed lower activations with correct, and higher activations with incorrect responses (Gur *et al.* 2007). While some authors hypothesized that a marked under-recruitment of the amygdala could be associated with schizophrenia

irrespective of the nature of the emotional task (Li *et al.* 2009), others regarded it as a phenomenon related to the disease state and to antipsychotic treatment (Rasetti *et al.* 2009).

A pivotal study by Taylor *et al.* (2006) described in healthy humans the neural correlates of response to emotional facial expressions in the offspring of families marked by harsh parenting with overt family conflict and deficient nurturing ('risky families', RFs). Participants with higher levels of adverse childhood experiences (ACEs) showed a reduced activation of the amygdala and a higher activation of the prefrontal cortex (PFC), which exerts an inhibitory effect on limbic structures stimulated by emotional tasks (Ochsner & Gross, 2005). Acute psychosocial stress causes a transient deactivation of limbic structures such as the amygdala (Pruessner *et al.* 2008); moreover, psychosocial stress experienced in childhood still correlates with lower responses of these structures in adult life (Taylor *et al.* 2006), thus possibly contributing to the negative relationship between ACEs and emotional skills in adult life (Repetti *et al.* 2002). Such a mechanism could contribute to the fMRI responses observed in patients with schizophrenia, also considering the suggested relationship between the breadth of early exposure to stress and later occurrence of physical and mental health disorders (Felitti *et al.* 1998), including schizophrenia (Collip *et al.* 2008; van Winkel *et al.* 2008). The issue of whether ACEs could influence fMRI responses to emotional paradigms has, however, not yet been addressed in patients with schizophrenia.

Here we studied the interaction of ACEs and adult schizophrenic psychopathology (undifferentiated subtype of chronic schizophrenia) on functional and structural correlates of automatic processing of negative facial expression.

## Method

### Sample, treatment and clinical assessment

We studied 20 in-patients (14 males and six females) affected by chronic schizophrenia, undifferentiated subtype [DSM-IV criteria, Structured Clinical Interview for DSM-IV (SCID-I) interview], according to the order of their admission to our hospital rehabilitation unit, with an age of 33.2 [standard deviation (s.d.) = 7.58] years, age at onset of illness of 23.7 (s.d. = 5.38) years, Positive and Negative Syndrome Scale total score 47.0 (s.d. = 6.74) [positive symptoms 22.1 (s.d. = 5.03), and negative symptoms 26.0 (s.d. = 5.51)]. All patients were on antipsychotic monotherapy [daily dosages: clozapine 160.7 (s.d. = 45.3), *n* = 9; haloperidol 4.5 (s.d. = 3.8), *n* = 5; risperidone 3.5 (s.d. = 0.6), *n* = 6; aripiprazole 12.5 (s.d. = 3.5), *n* = 2]. All the doses

of the antipsychotics were converted into chlorpromazine equivalents and mean administered doses were calculated; patients were being administered a mean dose of 271.77 (s.d. = 137.24) mg chlorpromazine equivalents of antipsychotic drugs). Serving as controls were 20 healthy age-matched participants [10 males and 10 females, aged 38.8 (s.d. = 10.86) years]. Exclusion criteria were mental retardation, substance abuse within the previous year, history of major physical illness and other psychiatric co-morbidity.

Severity of ACEs was rated on the Risky Families Questionnaire (RFQ) (Taylor *et al.* 2006) after fMRI scanning. The RFQ has been adapted from an instrument originally developed to assess the relationship of family stress to mental and physical health outcomes in adulthood (Felitti *et al.* 1998). Previous research validated this questionnaire against clinical interviews conducted and coded by trained clinical interviewers; the dual assessments (questionnaire and interview) demonstrated high agreement and reliability (Taylor *et al.* 2004). In the absence of validated cut-off values to discriminate 'higher' and 'lower' levels of ACEs, each group of 20 participants was then divided into two subgroups of 10 participants each by using median values as a discriminant between higher and lower scores of the RFQ (Taylor *et al.* 2006).

All data were collected on the same day. Severity of symptoms was rated on the Positive and Negative Syndrome Scale (Kay *et al.* 1987) in the morning. BOLD fMRI scanning took place in the afternoon. After complete description of the study to the subjects a written informed consent was obtained. The local ethical committee approved the study protocol.

#### *Cognitive activation paradigm and image acquisition*

We studied neural correlates of implicit emotional processing of facial affect expressions with a face-matching paradigm (Hariri *et al.* 2002). This paradigm has allowed researchers to define the effective connectivity of the amygdala with an extended regulatory network encompassing the cingulate, orbitofrontal, insular and dorsolateral PFC (Pezawas *et al.* 2005; Stein *et al.* 2007), and has already been tested in schizophrenia (Rasetti *et al.* 2009).

Four blocks of six pictures each representing human faces with fearful or angry expressions, interspersed with five blocks of six pictures of geometric shapes, were shown to the participants. Each picture is made up of two faces/shapes in the lower side and one in the upper part. Participants had to push a button on a response box to indicate which of the two images displayed in the lower side of the picture matched the one in the upper part (Hariri *et al.* 2002). Images were displayed for 4 s interleaved by a black screen.

Gradient echo and echo-planar images (EPIs) were acquired on a 3.0 T scanner (Gyrosan Intera; Philips, The Netherlands) using a six-channel sensitivity encoding (SENSE) head coil. For each functional run, 124 T2\*-weighted volumes were acquired using an EPI pulse sequence [repetition time (TR) = 3000 ms, echo time (TE) = 35 ms, flip angle = 90°, field of view = 230 mm, number of axial slices = 25, slice thickness = 5 mm, matrix size = 80 × 80 reconstructed up to 128 × 128 pixels]. Two dummy scans before fMRI acquisition allowed us to obtain longitudinal magnetization equilibrium. Total acquisition time was 6 min and 11 s. On the same occasion and using the same magnet a structural MRI (sMRI) scan for the analysis of voxel-based morphometry (VBM) was acquired using a T1-weighted magnetization-prepared rapid gradient-echo (MP-RAGE) sequence (TR 2500 ms, TE 4.6 ms, yielding 220 transversal slices with a thickness of 0.8 mm).

Images were computed, overlaid on anatomic images, and analysed using Statistical Parametric Mapping software (SPM5, Wellcome Department of Imaging Neuroscience, Institute of Neurology and the National Hospital for Neurology and Neurosurgery, UK). We realigned the scans to correct for head movement. Images were then normalized to a standard EPI template volume based on the Montreal Neurological Institute (MNI) reference brain, and smoothed using a 10-mm full-width at half-maximum (FWHM) isotropic Gaussian kernel. The evoked haemodynamic responses were modelled as a delta function convolved with a haemodynamic response function and its temporal derivative within the context of the general linear model.

Structural MRI data were processed with the VBM toolbox (VBM5.1) (University of Jena, Germany; <http://dbm.neuro.uni-jena.de/vbm/>) implemented in SPM5, which combines tissue segmentation, bias correction, and spatial normalization into a unified model. We used the optimized VBM procedure, which normalizes grey matter (GM) segmented images to a standard space by matching them to their template (Ashburner & Friston, 2005). The procedure yielded modulated GM normalized images: modulated parameters were used to test for voxelwise differences in the relative volume of GM by compensating for the effects of warping, to ensure that the total amount of GM in a region is the same before and after spatial normalization (Good *et al.* 2001). Images were then smoothed to an 8 mm FWHM isotropic Gaussian kernel.

#### *Data analysis*

Severity of ACEs was rated on the RFQ by summing the scores obtained on each of the 13 items (with each

score ranging from 1 to 15) in order to obtain a global score. In the absence of established cut-off values, the median of the distribution was then calculated for patients and controls and participants were divided into 'high RF' or 'low RF' based on their RFQ score being above or below the median score.

At the individual level we first compared (*t* test, threshold  $p < 0.001$ ) the face-matching condition with the shape-matching condition, thereby isolating regions that were engaged in the emotional processing of faces.

Contrasted images for each subject were then entered into a second-level analysis of variance with the severity of ACEs (high or low RFQ scores) and the diagnosis (schizophrenia or control) as factors, thus dividing participants into four groups (patients high/low RFQ; controls high/low RFQ). This procedure allowed for the main effects of (1) ACEs, and (2) diagnosis to be explored, and (3) for the regions where both factors significantly influenced the neural responses to the task to be identified (conjunction analysis, as implemented in the SPM5 statistical software package).

Using the Wake Forest PickAtlas software (Wake Forest University, USA; [www.fmri.wfubmc.edu](http://www.fmri.wfubmc.edu)), statistical maps were limited to GM and to *a priori* regions of interest (ROIs) based on previous reports about the effective connectivity of brain structures activated by our task (Stein *et al.* 2007) and on previous studies of the neural correlates of RFs (Taylor *et al.* 2006). The mask included the amygdala, hippocampus, anterior cingulate cortex [ACC; Brodmann's area (BA) 24 and 32] and PFC (BA 9, 10, 11, 12 and 46).

Second-level analyses were thresholded with a *p* value of 0.005 combined with a cluster size threshold of 10 voxels, which is the threshold used in the only previous study about neural correlates of ACEs (Taylor *et al.* 2006). Moreover, levels of significance were also corrected for multiple comparisons using a false discovery rate (FDR) threshold of  $p < 0.05$ .

For sMRI VBM analysis, modulated images were entered into a second-level analysis of variance for the group comparison of GM volume. We included total brain volume as a covariate to adjust for global atrophy and identify regions with differences that cannot be explained by the total GM differences among groups. The analysis was restricted to the same areas used for fMRI conjunction analysis. The statistical threshold was  $p < 0.05$ , FDR corrected for multiple comparison.

Given that antipsychotic medications have been reported to significantly influence both the functional connectivity between the amygdala and cingulate cortex in response to this task (Rasetti *et al.* 2009) and VBM estimates of GM volumes in schizophrenia

(McClure *et al.* 2006), we included medication load as a nuisance covariate in both fMRI and sMRI analyses. To estimate the medication load, we used the method of calculating chlorpromazine equivalent doses of the administered antipsychotics (BNF, 2008; Andreasen *et al.* 2010).

## Results

Patients reported significantly worse ACEs than controls [29.15, standard deviation (s.d.)=11.57 *v.* 20.15 (s.d.=4.77), Student's  $t=3.12$ , two-tailed  $p=0.003$ ]. Age and sex distribution did not significantly differ between patients and controls ( $p=0.06$  and  $p=0.55$ , respectively) and between participants with high and low RFQ scores ( $p=0.11$  and  $p=0.26$ ). Years of education differed between patients and controls [patients 11.6 (s.d.=2.6) *v.* controls 15.9 (s.d.=3.5) years,  $t=4.82$ ,  $p=0.0004$ ], but not between participants with high and low RFQ scores ( $p=0.23$ ).

### Functional imaging

Results of BOLD fMRI analyses are presented in Table 1 and plotted in Fig. 1.

Severity of ACEs significantly influenced neural responses of both patients and controls (Table 1, section A). The emotional faces paradigm was associated with reduced activations in the amygdala and hippocampus (Fig. 1*b*), and with greater activations in the ACC and PFC (Fig. 1*c*) in both patients and controls with higher RFQ scores compared with those with lower RFQ scores. Differences in the amygdala survived correction for multiple comparisons, and we found a trend toward this effect in the hippocampus ( $p=0.064$ , FDR corrected). The statistical significance of the observed differences was strengthened when considering medication load as a nuisance covariate.

The conjunction analysis (Table 1, section B; Fig. 1) showed that diagnosis and ACEs significantly influenced neural responses in the ROIs. Patients showed higher activations than controls in the right amygdala and hippocampus, and controls showed higher activations than patients in the PFC, at a threshold of  $p < 0.005$ , uncorrected. Differences observed in the hippocampus survived FDR correction for multiple comparison ( $p=0.011$ ) while a trend toward this effect was observed in the amygdala ( $p=0.051$ ). When considering the medication load as a nuisance covariate, the effect of diagnosis on the amygdala and hippocampus did not survive the threshold, but the statistical significance of the observed differences in the PFC and ACC was strengthened, with the ACC surviving FDR correction for multiple comparisons.

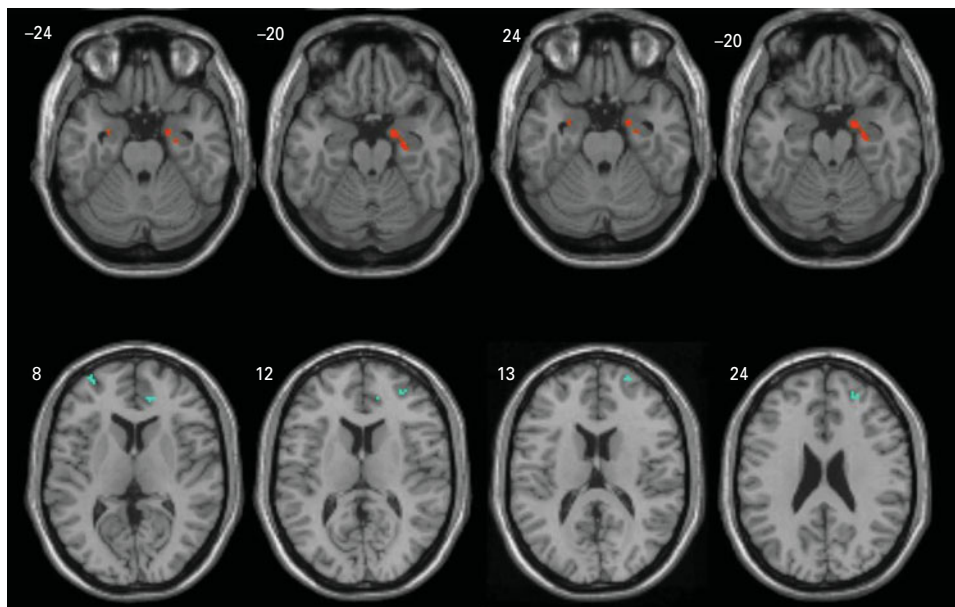
**Table 1.** Grey matter areas in a priori regions of interest showing a significant effect of diagnosis and ACEs on neural responses to the task, and effect of medication load<sup>a</sup>

Region	Direction of effects	Without considering medication load							Considering medication load						
		L/R	BA	Signal peak (x, y, z)	Z value	Cluster size (mm <sup>3</sup> )	Uncorrected p	FDR-corrected p	L/R	BA	Signal peak (x, y, z)	Z value	Cluster size (mm <sup>3</sup> )	Uncorrected p	FDR-corrected p
<b>(A) Grey matter areas showing a significant effect of ACEs on responses to the task in the whole sample</b>															
Amygdala	Low >high	R	–	20, –4, –26	2.87	216	0.002	0.046	R	–	20, –4, –26	2.89	216	0.002	0.045
Hippocampus	Low >high	R	–	28, –20, –18	2.98	144	0.001	0.064	R	–	28, –20, –18	3.07	160	0.001	0.055
Cingulate cortex	High >low	R	32	16, 44, 10	2.85	80	0.002	0.305	R	32	16, 44, 10	2.90	96	0.002	0.353
Prefrontal cortex	High >low	R	10	26, 60, 16	2.99	96	0.001	0.322	R	10	26, 60, 16	2.96	88	0.002	0.327
	High >low	R	10	34, 48, 10	2.96	96	0.002	0.322	R	10	34, 48, 10	2.94	80	0.002	0.327
	High >low	L	10	–32, 58, 8	2.87	104	0.002	0.322							
	High >low	R	10	26, 48, 26	2.87	88	0.002	0.322							
<b>(B) Conjunction analysis: grey matter areas showing a significant effect of both diagnosis and ACEs on neural responses</b>															
Amygdala	Low >high; P >C	R	–	28, –6, –20	2.96	224	0.002	0.051							
Hippocampus	Low >high; P >C	R	–	28, –14, –22	3.53	392	<0.001	0.011							
Prefrontal cortex	High >low; C >P	L	10	–20, 62, 0	3.42	120	<0.001	0.279	L	10	–30, 56, 6	3.43	656	<0.001	0.095
	High >low; C >P	L	10	–32, 56, 2	2.99	160	0.001	0.279	L	10	–16, 62, 24	3.16	104	0.001	0.095
	High >low; C >P								R	10	12, 46, 8	3.70	344	<0.001	0.095
	High >low; C >P								R	10	22, 54, 22	3.36	88	<0.001	0.095
	High >low; C >P								L	9	–6, 42, 18	3.41	416	<0.001	0.095
	High >low; C >P								R	32	16, 44, 8	3.76	672	<0.001	0.043
Cingulate cortex	High >low; C >P								L	32	–6, 44, 16	3.57	760	<0.001	0.043

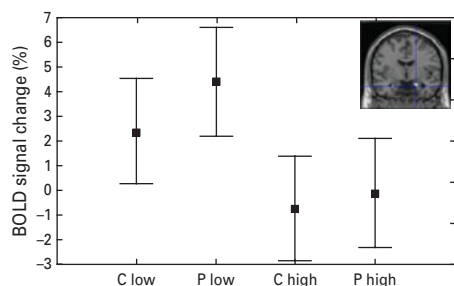
ACEs, Adverse childhood experiences; L, left; R, right; BA, Brodmann's area; FDR, false discovery rate; P, patients; C, controls; MNI, Montreal Neurological Institute.

<sup>a</sup> Data are shown for the main activations in each BA: lateralization (L/R); MNI coordinates (x, y, z) of voxels with higher Z values (signal peaks); cluster size (mm<sup>3</sup>); level of significance both uncorrected and corrected for multiple comparisons (FDR). Results are shown for activations surviving a threshold of  $p < 0.005$  in a cluster size  $> 10$  voxels. Direction of effect: high *versus* low ACEs, patients (P) *versus* controls (C).

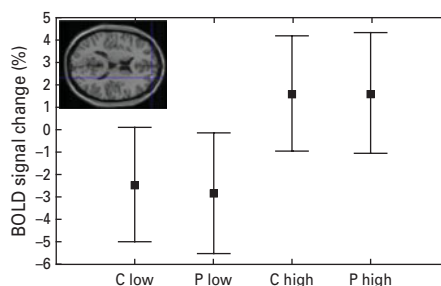
(a)



(b)



(c)



**Fig. 1.** Grey matter areas showing a significant effect of diagnosis and adverse childhood experiences (ACEs) on neural responses to the task (a), and direction and size effects of the observed differences in the amygdala (b) and superior frontal gyrus (c). (a) Lower reactivity is associated with higher ACEs (amygdala and hippocampus; red areas). Higher reactivity is associated with higher ACEs (prefrontal and cingulate cortex; blue areas). (b, c) Values are estimated regression coefficients for the tasks [percent of whole brain mean T2\* blood-oxygen-level-dependent (BOLD) signal]; whiskers are standard errors. Direction of effect: high *versus* low ACEs, patients (P) *versus* controls (C).

**Structural imaging**

Results of sMRI VBM analysis, considering medication load as a nuisance covariate, are presented in Table 2 and plotted in Fig. 2.

Patients showed a significant GM volume reduction compared with controls in the amygdala, hippocampus, ACC and PFC ( $p < 0.001$ , uncorrected). Differences observed in the bilateral amygdala and right hippocampus survived correction for multiple comparison (Table 2, section A).

Severity of ACEs was associated with greater GM volumes in the ACC and PFC ( $p < 0.001$ , uncorrected;  $p = 0.064$ , FDR corrected) (Table 2, section B).

Finally, the conjunction analysis showed highly significant effects of ACEs and diagnosis, all surviving FDR correction, of schizophrenia and ACEs on GM

volumes in the ACC and in multiple regions of the PFC (Table 2, section C). In these areas, (1) schizophrenia was associated with lower GM volumes, and (2) higher ACEs were associated with higher GM volumes (Fig. 2).

**Discussion**

This is the first study that combines BOLD fMRI and VBM sMRI to study emotional processing and ACEs in schizophrenia. Severity of ACEs and current chronic undifferentiated schizophrenia influenced neural responses to aversive facial expressions, and GM volumes in cortico-limbic structures implicated in emotional processing.

The pattern of activation in the ROIs extended knowledge about ACEs and the neural correlates of

**Table 2.** Brain areas showing a significant effect of diagnosis and ACEs on grey matter volumes<sup>a</sup>

Region	L/R	BA	Signal peak (x, y, z)	Z value	Cluster size (mm <sup>3</sup> )	Uncorrected <i>p</i>	FDR-corrected <i>p</i>
(A) Areas in which controls showed greater grey matter volumes compared with patients							
Amygdala	R	–	21, –7, –16	3.92	488	<0.001	0.003
	L	–	–20, –9, –16	3.67	76	<0.001	0.003
Hippocampus	R	–	30, –17, –17	4.00	359	<0.001	0.004
Anterior cingulate	R	32	9, 4, 46	3.65	63	<0.001	0.241
	R	24	5, 21, 24	3.32	30	<0.001	0.241
Prefrontal cortex	L	11	–16, 49, –21	3.49	67	<0.001	0.307
(B) Areas where higher ACEs were associated with higher grey matter volumes							
Prefrontal cortex							
Medial frontal gyrus	L	11	–14, 52, –19	4.27	120	<0.001	0.064
Middle frontal gyrus	R	11	25, 45, –11	3.67	208	<0.001	0.064
Orbital gyrus	L	11	–11, 54, –20	3.59	27	<0.001	0.064
Superior frontal gyrus	R	10	30, 55, –1	3.57	135	<0.001	0.064
Superior frontal gyrus	L	10	–25, 54, 6	3.44	44	<0.001	0.064
Middle frontal gyrus	R	10	30, 59, 23	3.44	46	<0.001	0.064
Inferior frontal Gyrus	L	47	–31, 14, –21	3.38	37	<0.001	0.064
Superior frontal gyrus	L	10	–20, 55, 27	3.34	74	<0.001	0.064
Middle frontal gyrus	R	11	38, 57, –12	3.34	44	<0.001	0.064
Anterior cingulate	R	24	3, 23, –4	3.44	192	<0.001	0.074
	L	24	–4, 23, –3	3.29	43	0.001	0.074
(C) Conjunction analysis: areas where both diagnosis and ACEs influence grey matter volumes							
Prefrontal cortex							
Superior frontal gyrus	L	11	–14, 52, –19	5.75	481	0.001	<0.001
Orbital gyrus	L	11	–11, 54, –21	4.86	126	<0.001	0.002
Superior frontal gyrus	L	10	–20, 54, 26	4.38	189	<0.001	0.004
Superior frontal gyrus	L	10	–25, 53, 7	4.15	126	<0.001	0.007
Middle frontal gyrus	R	11	25, 46, –13	4.03	247	<0.001	0.008
Middle frontal gyrus	L	9	–25, 40, 39	4.00	268	<0.001	0.009
Anterior cingulate	R	24	6, 31, 16	4.21	254	<0.001	0.014
	R	24	6, 33, –4	3.89	43	<0.001	0.014
	L	32	–5, 26, –8	3.44	31	<0.001	0.032
	L	24	–3, 30, 14	3.39	29	<0.001	0.021

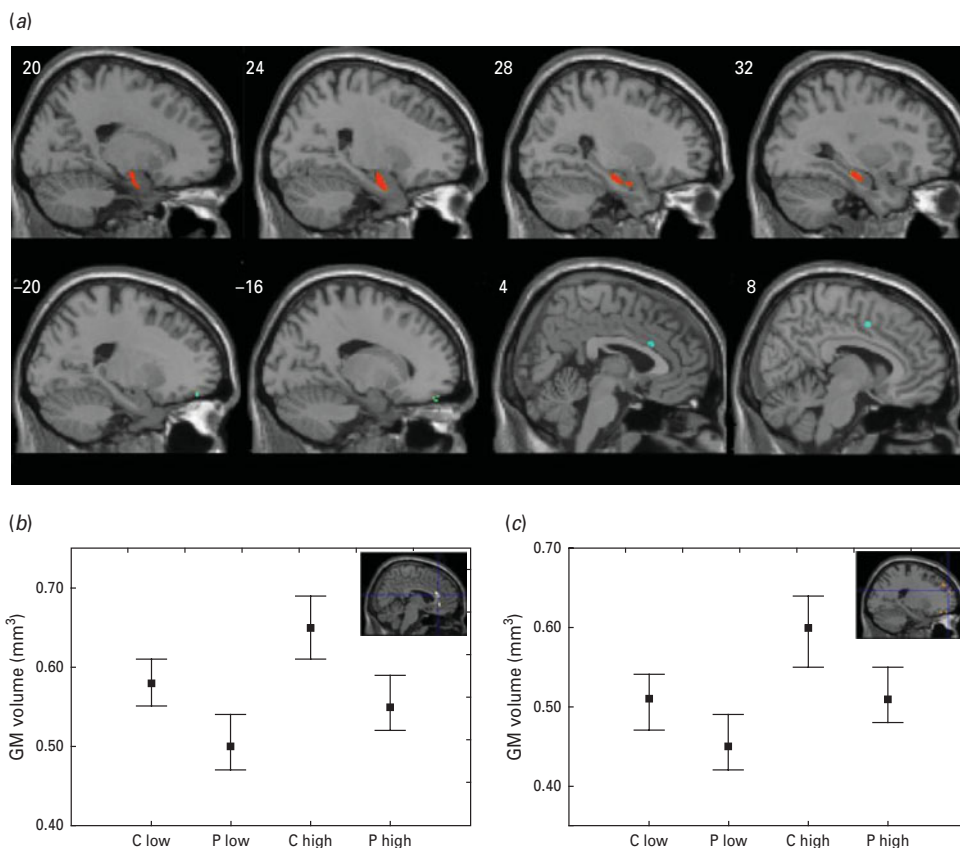
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<sup>a</sup>Data are shown for the main activations in each BA: lateralization (L/R), MNI coordinates (x, y, z) of voxels with higher Z values (signal peaks); cluster size (mm<sup>3</sup>); levels of significance (FDR). Results are shown for activations surviving a threshold of  $p < 0.001$  in a cluster size  $> 20$  voxels.

emotional processing. Confirming the findings of Taylor *et al.* (2006), who observed that normal subjects who had grown up in a RF environment had lower amygdala activations with passive observation of threatening emotional faces, we observed that higher ACEs were associated with lower responses in the amygdala and hippocampus of both healthy and schizophrenic participants. Moreover, Taylor *et al.* (2006) observed that higher ACEs led to higher activity in the PFC with an emotion-labelling task, and, using the same significance threshold, we observed a higher activity in the ACC and PFC with our matching task

(Stein *et al.* 2007). We then confirmed the hypothesis that growing up in RFs leads to persistently enhanced neural responses to threatening stimuli in brain regions associated with emotion control, and persistently lower responses in limbic structures associated with the experience of emotion.

This effect was observed in both patients and controls, but schizophrenia influenced neural responses; patients showed higher activations in the amygdala and hippocampus, and lower activations in the ACC and PFC. The effect of schizophrenia on fMRI responses in the amygdala and hippocampus was



**Fig. 2.** Brain areas showing a significant effect of diagnosis on grey matter (GM) volumes (a), and direction and size effects of the observed differences in the anterior cingulate gyrus (b) and the superior frontal gyrus (c). (a) The amygdala and hippocampus are shown in red. The prefrontal and cingulate cortex are shown in blue. (b, c) Values are estimated regression coefficients; whiskers are standard errors. Direction of effect: high *versus* low adverse childhood experiences, patients (P) *versus* controls (C).

largely linked with current medication status, thus confirming previous reports (Rasetti *et al.* 2009), but the effect on the PFC and ACC was independent of it.

The amygdala activates with bottom-up, stimulus-driven emotional processing (Wright *et al.* 2008), and neural responses in the amygdala correlate with the subjective experience of emotion (Liberzon *et al.* 2000; Taylor *et al.* 2003). Conversely, the PFC and ACC exert an inhibitory feedback on the amygdala in response to aversive stimuli (Pezawas *et al.* 2005). A deficit in the inhibitory function of these latter areas could then contribute to provide a neural basis for enhanced sensitivity to negative stimuli in schizophrenic patients (see Introduction).

Thus, severity of ACEs and current schizophrenia showed opposite effects on neural cortico-limbic responses to emotional stimuli. In agreement with literature data, patients reported significantly worse ACEs than controls, and this uncontrolled confounding factor could then have contributed to the discrepant reports in the field (see Introduction).

Similar considerations can be done for the observed effects of ACEs and schizophrenia on GM volumes. We observed that higher ACEs were associated with greater GM volumes in the ACC and PFC. Cognitive training rapidly results in increased GM volumes of areas activated by the cognitive tasks (Ceccarelli *et al.* 2009). It can be hypothesized that the increased reactivity of the ACC and PFC to emotional stimuli in participants with high ACEs could have led to higher GM volumes during their lifetime. Conversely, patients with schizophrenia showed a significant GM volume reduction in all the studied cortico-limbic regions, and the combination of the two factors (diagnosis and ACEs) significantly influenced GM volumes in the ACC and PFC.

The medial temporal lobe, ACC and PFC are key regions of structural difference in patients with schizophrenia compared with healthy subjects (Honea *et al.* 2005). A decreased volume of the amygdala and hippocampus has been reported both in adult and in child and adolescent schizophrenia (White *et al.* 2008; Benes,



2010). Longitudinal studies showed that medial temporal structures suffer aberrant changes in individuals at ultra-high risk for developing a psychotic illness (Wood *et al.* 2008), with normal volumes prior to the onset and reduced volumes after the onset of illness and in chronic schizophrenia (Velakoulis *et al.* 2006). Reduced GM volumes in the PFC have been extensively investigated in schizophrenia (Honea *et al.* 2005), and correlated with abnormal fMRI activations and neuropsychological deficits at working memory tasks (Callicott *et al.* 2000).

It is then tempting to speculate that the pattern of abnormal activations observed in schizophrenic patients could be due to these GM differences, which could be associated with neuropathological abnormalities including both quantitative (local volume changes; abnormal number, density and size of the neurons) and qualitative (cytoarchitectural upset, disarray of neuronal arrangement, and ectopic expression of neurons) abnormalities leading to marked changes in local neuronal microcircuitry (Iritani, 2007). An abnormal connectivity between brain areas involved in emotional processing has also been reported (Das *et al.* 2007), and could contribute to explain the observed abnormalities as well. The interaction of all these factors, and ACEs, could be central to the difficulties that patients experience when processing facial emotions (Li *et al.* 2009).

The present study was not aimed at defining the possible effects of early stress and of lifetime sensitization to stress in influencing the individual susceptibility to schizophrenia (Felitti *et al.* 1998; Collip *et al.* 2008; van Winkel *et al.* 2008), but we can conclude that ACEs should be carefully considered when studying the biological correlates of this illness. Some effects were more robust than others, and, using a more conservative statistical threshold than the study of Taylor *et al.* (2006) by applying FDR correction for multiple comparisons, we restricted significant fMRI effects of ACEs to the amygdala and hippocampus, and significant fMRI effects of combined ACEs and schizophrenia to the cingulate cortex, while all the effects of these factors on GM volumes survived this highly conservative threshold.

Limitations of the present study, which is retrospective and correlational in nature, also include issues such as generalizability, possible population stratification, a different level of education among groups, concomitant medications and their effects on the observed differences, non-drug naive, no placebo control, no evaluation for compliance, varying treatment periods, without consideration of gene-environment interactions, and technical issues such as slice thickness and smoothing kernel that could limit the specificity of the regional differences.

Finally, future longitudinal research is needed to chart the effects of schizophrenia and ACEs during the lifetime of the patients, and to assess the possible effect of their interaction on the development of psychopathology and its brain correlates.

### Acknowledgements

Our research unit received research grants from The Italian Ministry of University and Scientific Research, from the Italian Ministry of Health, from Trenta ore per la Vita Association, from Regione Lombardia, from the 7th Framework Program of the European Union, and from Janssen-Cilag.

### Declaration of Interest

None.

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