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Brain activation induced by psychological stress in patients with schizophrenia

M.N. Castro [a,b,c,d], M.F. Villarreal [a,d], N. Bolotinsky [d], E. Papávero [d], M.G. Goldschmidt [d], E.Y. Costanzo [c,d], L. Drucaroff [a,c,d], A. Wainsztein [d], D. de Achával [a,c,d], J. Pahissa [d], K.-J. Bär [e], C.B. Nemeroff [f], S.M. Guinjoan [a,c,d,g,*]

[a] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET), Buenos Aires, Argentina
[b] Department of Physiology, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
[c] Department of Mental Health, School of Medicine, University of Buenos Aires, Buenos Aires, Argentina
[d] Fundación Lucha contra Enfermedades Neurológicas de la Infancia (FLENI), Buenos Aires, Argentina
[e] Department of Psychiatry, Universitätsklinikum Jena, Friedrich-Schiller-Universität, Jena, Germany
[f] Department of Psychiatry and Behavioral Sciences, Leonard M. Millier School of Medicine, University of Miami, Miami, United States
[g] Department of Neurophysiology, School of Psychology, University of Buenos Aires, Buenos Aires, Argentina

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ABSTRACT

Environmental influences are critical for the expression of genes putatively related to the behavioral and cognitive phenotypes of schizophrenia. Among such factors, psychosocial stress has been proposed to play a major role in the expression of symptoms. However, it is unsettled how stress interacts with pathophysiological pathways to produce the disease. We studied 21 patients with schizophrenia and 21 healthy controls aged 18 to 50 years with 3T-fMRI, in which a period of 6 min of resting state acquisition was followed by a block design, with three blocks of 1-min control-task, 1-min stress-task and 1-min rest after-task. Self-report of stress and PANSS were measured. Limbic structures were activated in schizophrenia patients by simple tasks and remained active during, and shortly after stress. In controls, stress-related brain activation was more time-focused, and restricted to the stressful task itself. Negative symptom severity was inversely related to activation of anterior cingulum and orbitofrontal cortex. Results might represent the neurobiological aspect of hyper-reactivity to normal stressful situations previously described in schizophrenia, thus providing evidence on the involvement of limbic areas in the response to stress in schizophrenia. Patients present a pattern of persistent limbic activation probably contributing to hypervigilance and subsequent psychotic thought distortions.

1. Introduction

In most cases of schizophrenia, heritability seems to result from a large number of predisposing single nucleotide polymorphisms, each with a very small contribution to increased risk, and also very prevalent in the general population (Schizophrenia Working Group, 2014). This pattern of heritability probably explains the observation that the environment has long been recognized as a critical factor, at different developmental steps, for the expression of the behavioral and cognitive phenotypes of the disease. Among such environmental factors, psychosocial stress is a major determinant in the onset and worsening of schizophrenia symptoms. For example, Myin-Germeys and van Os (2007) observed an increased emotional reactivity to daily stress in patients with schizophrenia and first-degree relatives, whereas Castro et al. (2008, 2009) demonstrated the presence of a cardiac autonomic response to acute mental stress which in contrast to healthy controls, was protracted beyond stressful stimulus cessation. These observations suggest that vulnerability to stress may be a trait marker of schizophrenia. However, it is unsettled how stress interacts with pathophysiological pathways related to gene variants to produce the disorder, and most research has focused on neurotransmitter systems—especially dopamine (e.g. Lataster et al., 2014). In healthy persons, fMRI and PET studies have identified several cortical and subcortical areas as being activated or deactivated in response to stress (e.g., Dedovic et al., 2009a; Pruessner et al., 2008). Stressors which require the completion of demanding and uncontrollable cognitive challenges in the context of negative social evaluation induce increased activity at the medial prefrontal cortex (Urry et al., 2006; Kern et al., 2008), anterior cingulate cortex—which may be of particular importance for generating autonomic responses (Critchley et al., 2000a,b, 2005; Critchley, 2005), insula—which probably works together with anterior cingulum, as both are components of a system underlying self awareness (Medford and Critchley, 2010) and deactivation of the hippocampus–amygdala complex (Kern et al., 2008), which probably results in disinhibition of the hypothalamus; the latter in turn commands hyphotalamic-pituitary and autonomic responses (McEwen and Gianaros, 2010). Some authors

* Corresponding author at: FLENI, Dept. of Mental Health, UBA School of Medicine, Montañeses 2325 8th Floor, C1428AQK Buenos Aires, Argentina.
E-mail address: sguinjoan@fleni.org.ar (S.M. Guinjoan).

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have used a variety of tests to induce stress by generating a social evaluative threat combining an arithmetic task with a social evaluative component (Dedovic et al., 2005). They found a deactivation of diverse limbic system components, including hippocampus, hypothalamus, medial orbitofrontal cortex, and anterior cingulum, concluding that such deactivation would permit the initiation of the stress response by the hypothalamic–pituitary system (Dedovic et al., 2009a; Pruessner et al., 2008). However, we are not aware of studies systematically probing the functional brain correlates of acute stress in patients with clinically stable schizophrenia using fMRI techniques, which have a more favorable time definition than PET, thus permitting the observation of brain activity changes during, and immediately after psychological stress. This is indeed an important step to fully characterize the neurobiological mechanisms operating the diathesis-stress model of disease, formulated three decades ago (Nuechterlein and Dawson, 1984; Kendler et al., 2004; Kendler and O'Donovan, 2014). On the basis of the aforementioned observations made by our group and others on psychological and autonomic reactions to acute stress, we hypothesized that patients would have a pattern of brain activation during acute stress that would be similar to healthy controls, but that in contrast to them, this pattern would persist beyond stimulus termination, hence providing a basis for both the subjective stress experience and its bodily correlates. To test this hypothesis, we used a functional MRI paradigm of stress induction, and compared brain activation in patients with schizophrenia and healthy subjects during and after acute mental stress. In addition to performing a whole-brain analysis of functional images, and based on previous findings about this topic, we also focused on five regions of interest relevant to stress physiology (Medford and Critchley, 2010; Dedovic et al., 2009a; Kern et al., 2008; Pruessner et al., 2008; LeDoux, 1995), namely: amygdala, hippocampus, anterior cingulate, orbitofrontal cortex, and insula.

2. Methods and materials

All participants were assessed at the Psychiatry Section, FLENI Institute, Buenos Aires. All participants gave written informed consent as approved by the local bioethics committee, performed in accordance with the ethical standards set by the 1964 Declaration of Helsinki.

2.1. Participants

2.1.1. Patients (SZ)

Psychiatry outpatients were invited to participate in the study if they (a) received a DSM-IV-TR (American Psychiatric Association, 1994) diagnosis of schizophrenia (any subtype), confirmed with a Composite International Diagnostic Interview (Robins et al., 1988) administered by a consultant psychiatrist (SMG or MNC), (b) were aged 18–65 years, and (c) had been on the same medications for at least two weeks. Exclusion criteria: (a) misuse or addiction to illegal substances in the previous 6 months, (b) active symptoms having warranted antipsychotic dose adjustment or admission to the hospital, day hospital, or intensive outpatient treatment, in the preceding 2 weeks, (c) a history of mental retardation, or (d) a history of active cardiovascular symptomatology and head trauma resulting in loss of consciousness. We obtained a structural MRI to exclude any underlying anatomical abnormality. Twenty-one SZ (9 females, aged 29 ± 7 years) were recruited for this study.

2.1.2. Healthy controls (HC)

Healthy volunteers were recruited from local community; they were offered no financial compensation for their participation. Exclusion criteria: (a) the lifetime presence of any DSM-IV-TR Axis I anxiety, mood, or psychotic disorder diagnosis as detected by a psychiatric interview with a consultant psychiatrist and (b) a medication history of antidepressants, antipsychotics, or mood stabilizers. Twenty-one subjects (8 females, aged 27 ± 7 years, range years) were studied.

2.2. Procedures

2.2.1. Screening tests

All participants were screened for premorbid intelligence with the Word Accentuation Test (WAT; Del Ser et al., 1997; de Achával et al., 2012) and for depressive symptoms with the Hamilton depression test (HAM-D; Hamilton, 1960). All patients were evaluated using Positive and Negative Symptoms Scale (PANSS; Kay et al., 1987) to measure psychotic symptom severity.

2.2.2. fMRI stimuli

All subjects were evaluated between 17:00 and 20:00 h. We used a stress paradigm based on previous studies (Ewing, 1992; Dedovic et al., 2005). A period of 6 min of resting state (PRE) acquisition was followed by a block-design which had three blocks of 1-minCONTROL-task, 1-minSTRESS-task and 1-minrest post-stress (POST). CONTROL-task consisted in a one-digit sum of three terms, which had a very low difficulty level (Fig. 1). STRESS-task consisted of two subtractions of two-digit, or one subtraction plus one sum of two-digit, therefore making it more stressful. During stress-task, the screen displayed the remaining time with a countdown timer. The allocated time was calculated using information from a previous training session (done inside the fMRI device), from which we subtracted 20% of allotted time to generate more stressful conditions; thus this time was specific to each subject. Participants picked their response from a row of numbers (from 0 up to 9) using a two-button response box. With one button, they moved the cursor along the numbers, and with the other button they selected the chosen number; equations were designed so that all correct results were between 0 and 9. During POST-task the screen displayed a black fixation cross in a white background. All participants were advised to perform as accurately as possible and told that the evaluator would be controlling their responses, so as to generate a social negative evaluation.

We evaluated the performance during each condition measured as the percentage of correct responses. After scan, subjects were required to report a scale of subjective stress, with items including self-report of stress and anxiety level during resting inside scan and during the stress task, the level of effort, task difficulty and frustration generated by the stress task (on a scale of 1 to 10; adapted from Wang et al., 2005).

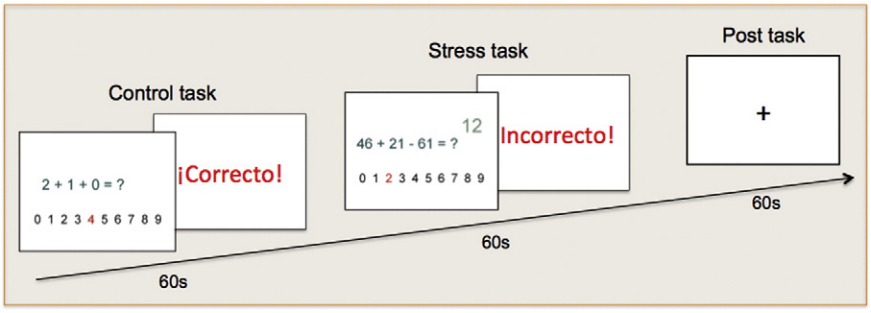

Fig. 1. Block design paradigm with three blocks of 1-minute CONTROL task, 1 minute STRESS task and 1 minute rest after task (POST).

2.2.3. fMRI data acquisition

MRI data were acquired on a 3T General Electric HDx scanner with an 8 channel head coil. Change in blood-oxygenation-level-dependent (BOLD) T2* signal was measured using a gradient echo-planar imaging (EPI) sequence. Thirty-three contiguous slices were obtained in the AC–PC plane (TR 2 s, TE 30 ms, flip angle 90°, FOV 24 cm, 64 × 64 pixels per inch matrix, voxel size = 3.75 × 3.75 × 4). A structural MRI was obtained with the fast SPGR-IR sequence (166 slices, 1.2-mm thick slices, TR 7.256 ms, TE 2.988 ms, flip angle 8°, FOV 26 cm, 256 × 256 matrix). Two sessions of 200 (PRE) and 280 (block design paradigm: CONTROL-STRESS-POST) volumes were taken per subject.

2.3. Statistical analysis

2.3.1. Analysis of demographical data

Discrete variables in patients and controls were compared using a chi-square test. Continuous variables were compared with an independent-sample *t* test. In all cases, the tests applied were two tailed and significance was assumed at $\alpha < 0.05$. All statistical analysis was performed with the SPSS version 18.0 software (SPSS Inc.).

2.3.2. fMRI analysis

2.3.2.1. Imaging processing. Image processing was carried out using SPM 8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks Inc., Sherborn, MA, USA). Slice-timing correction was applied to each volume. The imaging time series was realigned to the first volume. The voxel size used was 3.75 × 3.75 × 4mm, which was then normalized volumes to the stereotactic space of Talairach and Tournoux (1998) using the Montreal Neurological Institute reference brain to the default size of 2 × 2 × 2mm^3 (Ashburner and Friston, 1999), as previously employed by our group and others when dealing with cognitive and emotional paradigms (e.g., Goldschmidt et al., 2014; Mascali et al., 2015; Costanzo et al., 2015). Normalized volumes were spatially smoothed by an isotropic Gaussian kernel of 8 mm at full width half-maximum (Friston et al., 2000). We performed a rigid body affine transformation to obtain the six head motion regressors (Ashburner and Friston, 1999). We used as regressors of no interest the three parameters of translation and three of rotation (Ashburner and Friston, 1999).

2.3.2.2. Image statistical analysis. Individual analysis was computed using the general linear model including the experimental conditions (CONTROL, STRESS and POST) and the baseline condition (PRE). The design matrix also included correction for head movements as regressors of no interest. The effects were modeled using a canonical hemodynamic response function convolved with a boxcar to create regressors of interest. We evaluated linear contrasts: STRESS > CONTROL, POST > CONTROL, CONTROL > PRE, STRESS > PRE and POST > PRE.

Differences between and within-groups were analyzed with a 2 × 3 ANOVA test (GROUP × CONDITION) for patients vs. controls during each condition.

We used a statistical threshold FWE corrected $p < 0.05$.

2.3.2.3. ROI analysis and relationship with clinical measures. We explored activation in specific regions based upon the study hypotheses, namely a) previous reported areas related to stress response (Dedovic et al., 2005; McEwen and Gianaros, 2010; Critchley et al., 2003; Williamson et al., 1997; Soufer et al., 1998; Harper et al., 1998) and to cognitive task (Hugdahl et al., 2004), b) our previous studies (Castro et al., 2008, 2009) and c) the results of the experimental CONDITION effect. These ROIs were defined from automated anatomical labeling (AAL) atlas. We studied hippocampus, amygdala, anterior cingulate and orbitofrontal cortex, and insula. We performed a division for head, body and tail of hippocampus using a validated protocol (Pruessner et al., 2000).

We made a small volume correction in STRESS > CONTROL and POST > CONTROL contrast between groups, and in linear contrasts of each group. All results were FWE corrected $p < 0.05$.

We extracted the beta signal from each ROI using MATLAB processing to analyze them within and between-groups. We made a multivariate ANOVA to compare all ROIs between-groups and repeated-measures ANOVA to compare each experimental condition within-group, followed by a Bonferroni post hoc test. The location of brain activity was reported in the MNI system. Significance was assumed at $p < 0.05$.

We made a Pearson correlation analysis between beta signal and clinical measures. The results were two-tailed and set at 0.05.

3. Results

Table 1 shows demographic and clinical characteristics of participants. Both groups were similar regarding age and gender. Patients had fewer years of education and lower premorbid intelligence than control subjects ($p < 0.001$; Table 1). All patients were treated with atypical antipsychotics (Table 1); average chlorpromazine equivalent daily dose (Woods, 2003) was 575.16 mg. All subjects were right-handed. Patients had worse performance in the arithmetic tasks (Table 1).

3.1. ANOVA results

Within the ANOVA test the GROUP influence on brain activation showed significant differences for bilateral supplementary motor area, superior frontal and fusiform gyri, left-superior parietal gyrus and occipital areas ($F = 13.37$; FWE corrected $p < 0.05$).

The CONDITION effect showed statistic significance for bilateral thalamus, hippocampi, angular gyri, anterior cinguli, orbitofrontal cortex, insulae, superior frontal gyri and middle frontal gyri, supplementary motor area, pre- and post-central gyri, superior and inferior parietal gyri, lingual gyri, precuneus, inferior temporal and fusiform gyri, right-parahippocampus, pons, bilateral cerebellar hemispheres and vermis ($F = 10.14$; FWE corrected $p < 0.05$).

The GROUP × CONDITION interaction did not show corrected statistic significance.

Table 1
Clinical and demographic characteristics.

	Patients ($n = 21$)	Controls ($n = 21$)	Statistic	*p*
Age (years)	29 ± 7	27 ± 7	$t = 1.067$	0.292
Female	9 (42.9)	8 (38.1)	$X^2 = .099$	0.753
Education (years)	13 ± 2	16 ± 3	$t = -4.187$	0.000
Smoke	7 (33.3)	5 (23.8)	$X^2 = .467$	0.495
WAT	30 ± 7	34 ± 5	$t = -2.066$	0.045
HAM-D score	6 ± 4	3 ± 2	$t = 3.627$	0.001
Performance	37 ± 16	53 ± 18	$t = -3.003$	0.005
Age at onset	22 ± 5	–	–	–
First episode	3 (14.3)	–	–	–
Disease duration (years)	7 ± 5	–	–	–
PANSS total score	81 ± 31	–	–	–
Positive subscale	17 ± 7	–	–	–
Negative subscale	25 ± 10	–	–	–
Novel antipsychotics				
Risperidone	10 (47.6)	–	–	–
Olanzapine	3 (14.3)	–	–	–
Clozapine	5 (23.8)	–	–	–
Quetiapine	5 (23.8)	–	–	–
Selective serotonin reuptake inhibitor	5 (23.8)	–	–	–
Benzodiazepine	6 (28.6)	1 (4.8)	$X^2 = 4.286$	0.038

WAT: Word Accentuation Test; HAM-D: Hamilton depression score; Performance = percentage of correct responses. All values are showed as n (%) or mean ± SD.

3.1.1. Within-group comparisons

A within-group analysis of HC during stress-task revealed activation of bilateral orbitofrontal cortex, anterior cinguli, superior and middle frontal gyri, supplementary motor area, insulae, superior and inferior parietal gyri, precuneus and angular gyri, right-hippocampus and parahippocampus, right-medial superior frontal gyrus and supramarginal gyrus, left-inferior frontal gyrus, left-pre- and post-central area, occipital areas and pons (STRESS > CONTROL; Fig. 2A, Table 2, FWE corrected $p < 0.05$). However, SZ did not display activation of head of hippocampus, parahippocampus or insula during the same contrast (STRESS > CONTROL; Fig. 2B, Table 2, FWE corrected $p < 0.05$). Their t map showed bilateral activation of superior and middle frontal gyri, left-inferior frontal gyrus, left-precentral area, left-superior and inferior parietal gyrus, and occipital areas during stress task (STRESS > CONTROL; Fig. 2B, Table 2, FWE corrected $p < 0.05$).

During post-period HC displayed activation of bilateral anterior cinguli, orbitofrontal cortex and superior frontal gyri, left hippocampus, parahippocampus, and amygdala (POST > CONTROL; Fig. 2C; Table 2; FWE corrected $p < 0.05$). However, SZ showed, during the same contrast, activation of bilateral precuneus and left angular gyrus (POST > CONTROL; Fig. 2D, Table 2, FWE corrected $p < 0.05$).

3.1.2. Between-group comparisons

We found differences in activation (SZ < HC) during STRESS > CONTROL contrast of bilateral anterior cinguli (Fig. 3). During CONTROL > PRE we found greater activation in patients (SZ > HC) in the left hippocampus, insula, orbitofrontal cortex, superior temporal pole, middle cingulate cortex and precuneus. STRESS > PRE contrast also showed greater activation in patients (SZ > HC) of left precuneus. Once the stress task ceased (POST > PRE) we observed activation differences (SZ > HC) in the right angular gyrus and supplementary motor area.

3.2. ROI analysis

HC presented greater activation during stress than control task in both right ($p = 0.032$) and left-amygdala ($p = 0.013$, Fig. 4A, [(STRESS > PRE) > (CONTROL > PRE)]), as well as lower activation than post-period in the left amygdala ([(STRESS > PRE) > (POST > PRE)]; $p = 0.017$, Fig. 4A). However, SZ did not present significant differences in this regard. Comparing both groups we found a greater activity in left amygdala during control-task in SZ compared with HC (SZ > HC; $F = 5.096$, $p = 0.030$, Fig. 4A).

In HC, the head of the right ($p = 0.016$) and left hippocampus ($p = 0.012$, Fig. 4B) had higher activation during stress-task compared with control-task [(STRESS > PRE) > (CONTROL > PRE)], and lower activation than post-period in the left head of the hippocampus ([(STRESS > PRE) > (POST > PRE)]; $p = 0.030$, Fig. 4B). However, SZ did not present significant differences in these analyses.

HC presented higher activation during stress compared with control task at the right ([(STRESS > PRE) > (CONTROL > PRE)]; $p < 0.001$), and left anterior cingulum ($p < 0.001$; Fig. 4C), as well as lower activation than post-period [(STRESS > PRE) > (POST > PRE)] in the left anterior cingulum ($p = 0.019$, Fig. 4C). Again, SZ did not present significant differences in these comparisons.

In HC, the left insula showed greater activation during stress compared with both control and post-task ($p = 0.022$ for [(STRESS > PRE) > (CONTROL > PRE)]; $p = 0.039$ for [(STRESS > PRE) > (POST > PRE)]), and higher activation of the right insula compared to control task [(STRESS > PRE) > (CONTROL > PRE)], $p = 0.040$). However, SZ presented greater activation of left-insula during stress compared with post period ([(STRESS > PRE) > (POST > PRE)], $p = 0.048$), and higher activation of right insula during control compared with stress task ([(CONTROL > PRE) > (STRESS > PRE)], $p = 0.019$) and during stress compared with post-task ([(STRESS > PRE) > (POST > PRE)], $p = 0.038$).

3.3. Correlation analyses

SZ showed an inverse correlation between negative symptom severity and anterior cinguli activation during both stress and post-stress period for left (STRESS > PRE: $r = -0.478$, $p = 0.028$, POST > PRE: $r = -0.445$, $p = 0.043$; Fig. 5A), and during all periods for the right anterior cingulum (CONTROL > PRE: $r = -0.490$, $p = 0.024$; STRESS > PRE: $r = -0.503$, $p = 0.020$; POST > PRE: $r = -0.527$, $p = 0.014$; Fig. 5B), as well as the right orbitofrontal cortex during post-stress period (POST > PRE: $r = -0.439$, $p = 0.046$; data not shown).

SZ showed a negative correlation between performance and right amygdala during stress task (STRESS > CONTROL; $r = -0.554$, $p = 0.009$; Fig. 5C) as well as the left ($r = -0.502$, $p = 0.021$) and right head of the hippocampus during the same period ($r = -0.535$, $p = 0.013$; Fig. 5D).

Finally, SZ showed a positive correlation between the frustration item of stress questionnaire and right amygdala activity during stress (STRESS > PRE contrast; $r = 0.454$, $p = 0.039$; Fig. 5E) as well as the bilateral head of hippocampi ($r = 0.486$, $p = 0.026$ for left; $r = 0.457$, $p = 0.037$ for right; Fig. 5F) during the same contrast.

These relationships were not present in HC.

4. Discussion

The main finding of this study is that the pattern of brain activation in relation to acute mental stress is different in patients with schizophrenia and healthy subjects, in that activation of limbic structures

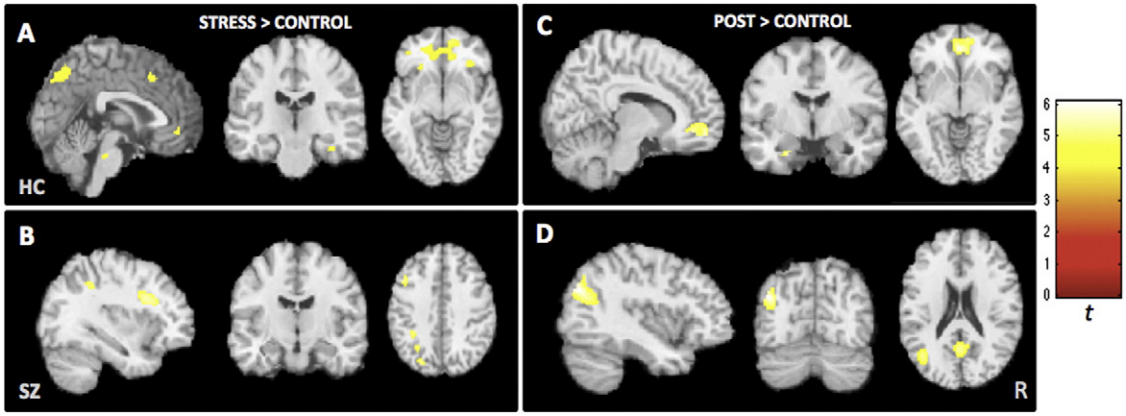

Fig. 2. Brain activation during mental stress (STRESS > CONTROL contrast) in A. healthy subjects (HC; $x = -2, y = -24, z = -2$) and B. patients with schizophrenia (SZ; $x = -38, y = -14, z = 46$); and during POST > CONTROL contrast in C.HC ($x = 6, y = -6, z = 0$), and D.SZ ($x = -44, y = -76, z = 26$). ANOVA analysis within group. All results FWE corrected $p = 0.05$.

Table 2
Brain activity within group.

Healthy controls						Patients with schizophrenia					
Regions	Cluster size (Voxels)	Coordinates			*T* value	Regions	Cluster size (Voxels)	Coordinates			*T* value
		x	*y*	*z*				*x*	*y*	*z*	
STRESS > CONTROL contrast						STRESS > CONTROL contrast					
Activations						Activations					
L inferior frontal gyrus	2358	−42	18	28	7.04	L precentral area	671	−44	8	32	6.09
L middle frontal gyrus						L middle frontal gyrus					
L precentralgyrus						L inferior frontal gyrus					
L postcentralgyrus						L superior frontal gyrus	238	−26	6	70	5.81
L inferior parietal gyrus	1368	−40	−54	44	6.45	R superior frontal gyrus	36	28	4	62	5.06
L superior parietal gyrus						R middle frontal gyrus					
B precuneus						L superior parietal gyrus	125	−28	−78	46	5.04
L angular gyrus						L middle occipital gyrus					
L middle occipital gyrus						L inferior parietal gyrus	48	−36	−44	44	5.01
L superior occipital gyrus											
R superior frontal gyrus	367	30	−2	68	6.16						
R middle frontal gyrus											
B superior frontal gyri	510	10	50	0	5.92						
R medial superior frontal gyrus											
B anterior cingulum											
B orbitofrontal cortex											
R inferior parietal gyrus	111	34	−44	38	5.54						
R angular gyrus											
R supramarginal gyrus (TPJ)											
B supplementary motor area	92	−4	16	48	5.18						
R insula	44	30	26	−2	5.11						
R hippocampus	35	34	−26	−18	5.11						
R parahippocampus											
R superior occipital gyrus	42	22	−76	46	5.07						
R superior parietal gyrus											
R Precuneo											
L Insula	69	−30	22	−6	4.91						
Pons	27	−6	−28	−26	4.41						
POST > CONTROL contrast						POST > CONTROL contrast					
Activations						Activations					
B anterior cingulum	466	−4	42	−2	6.55	L angular gyrus	446	−46	−76	34	6.82
B orbitofrontal cortex						B precuneus	530	−2	−60	30	6.05
B medial superior frontal gyrus											
L hippocampus	74	−26	−8	−24	5.84						
L parahippocampus											
L amygdala											

Brain regions with significant increase BOLD contrast signal within group comparison. FWE corrected $p > 0.05$ for all results; L = left; R = right; B = bilateral.

typically associated with stress and arousal, in schizophrenia is triggered by simple tasks and persist during, and shortly after stress. In healthy individuals, brain activity is more time-focused, and restricted to stressful tasks as probed in the present paradigm. These results are coincident with psychosocial research showing that patients with schizophrenia have an exaggerated reactivity to stimuli not considered

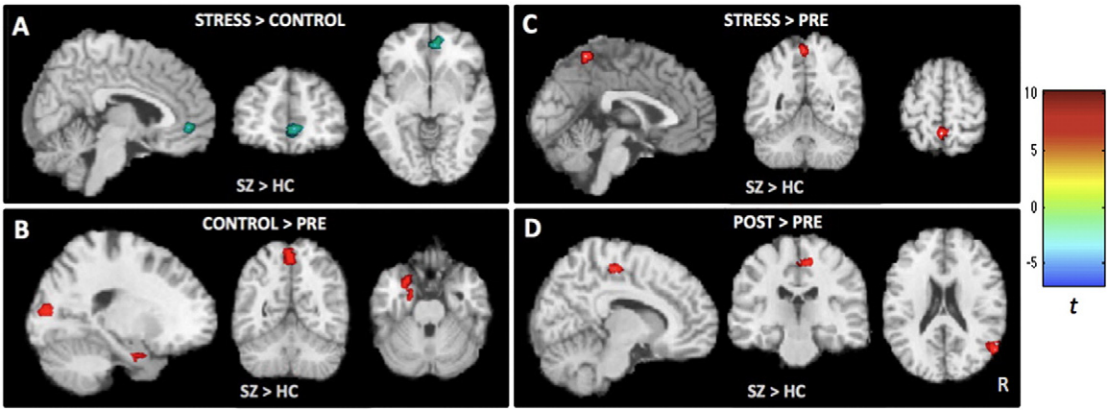

Fig. 3. A. Brain deactivation during STRESS > CONTROL contrast ($x = 2, y = 44, z = 0$), and brain activations during B. CONTROL > PRE contrast ($x = -24, y = -58, z = -22$), C. STRESS > PRE contrast ($x = -4, y = -54, z = 68$), and D. POST > PRE contrast ($x = 6, y = -26, z = 26$) in patients with schizophrenia (SZ) vs. control subjects (HC) comparison (SZ > HC). All results FWE corrected $p < 0.05$ across small volume correction.

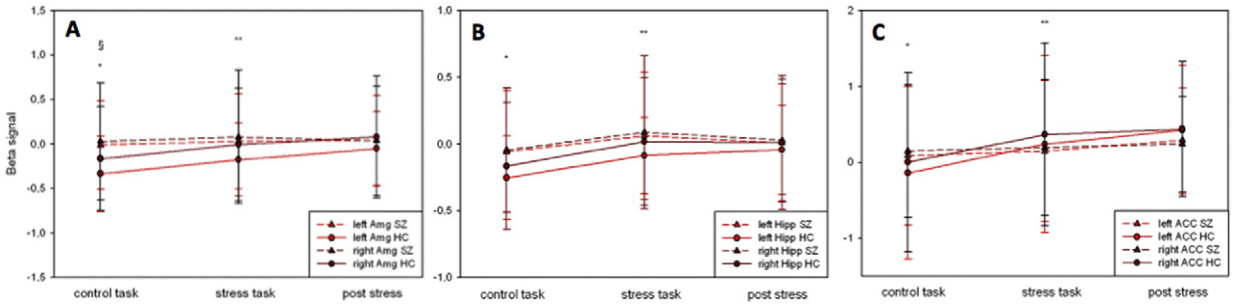

Fig. 4. Activation of ROIs in both groups. A. Activation of amygdala (Amg) during all conditions in patients with schizophrenia (SZ) and healthy controls (HC).$^{*}p = 0.032$ vs. stress task for right-Amg, and $^{**}p = 0.013$ and 0.017 vs. control and post-period respectively for left-Amg in HC (repeated measures ANOVA followed by Bonferroni post hoc); $^{\S}p = 0.030$ vs. HC for left Amg ($F = 5.096$; MANOVA analysis); B. Activation of hippocampus head (Hipp) during all conditions in SZ and HC.$^{*}p = 0.016$ vs. stress task for right-Hipp and $^{**}p = 0.012$ and 0.030 vs. control and post period respectively for left-Hipp in HC (repeated measures ANOVA followed by Bonferroni post hoc); C. Activation of anterior cingulate cortex (ACC) during all conditions in SZ and HC. $^{*}p = 0.000$ vs. stress period for right-ACC, and $^{**}p = 0.000$ and 0.019 vs. control and post period respectively for left-ACC in HC (repeated measures ANOVA followed by Bonferroni post hoc).

stressful in normal conditions, as well as a protracted neurophysiological response to truly stressful stimuli (Myin-Germeys and van Os, 2007; Castro et al., 2008). Moreover, the present results can be understood as a potential explanation for previous observation demonstrating poorly time-focused autonomic activation, with protracted autonomic nervous system activity persisting beyond stimulus termination (Castro et al., 2008). Indeed, in the present study we observed that mental arithmetic stress evokes increased activity in brain areas related to hierarchical control of autonomic activity in healthy individuals, but such areas are already active in patients during a simple control task, and remain activated during a mental arithmetic task. This observation might represent a neurobiological signature of hyperreactivity to normal stressful situations previously described in patients with schizophrenia (Myin-Germeys and van Os, 2007).

It is known that stressors induce a response effected by the autonomic nervous system (ANS) and the hypothalamo-pituitary-adrenal axis (HPA axis; McEwen and Gianaros, 2010; McEwen, 2000; Mason, 1959, 1975). Some previous works on this topic have found that in HC some areas linked to the limbic system including regions of medial temporal lobe, insular lobe and prefrontal cortex (MacLean, 1949, 1952; Catani et al., 2013; Rolls, 2015) are activated during stress (Critchley et al., 2000a,b). Thus, the amygdalo-hippocampal complex, orbitofrontal cortex, anterior cingulum and insula have been shown to be activated by both mental and physical tests, which are useful to evoke an autonomic stress response (Critchley et al., 2003; Williamson et al., 1997; Soufer et al., 1998; Harper et al., 1998).

Stressors which require the completion of demanding and uncontrollable cognitive challenges in a context of negative social evaluation, such as the Trier Social Stress Test (TSST) induce increased activity of the medial prefrontal cortex (Kern et al., 2008; Urry et al., 2006), anterior cingulum (which may be of particular importance for generating autonomic cardiovascular responses; Critchley et al., 2000a,b, 2005; Critchley, 2005), insula (which probably works together with anterior cingulum, as both are components of a system underlying self awareness; Medford and Critchley, 2010), and deactivation of the hippocampal-amygdala complex (Kern et al., 2008), probably to disinhibit the hypothalamus which commands the HPA and ANS (McEwen and Gianaros, 2010) responses. Dedovic et al. (2005) have used a variety of tests to induce stress by generating a social evaluative threat combining an arithmetic task and a social evaluative component, such as the Montreal Imaging Stress Task (MIST). They found a deactivation of the limbic system including hippocampus, hypothalamus, medial orbitofrontal cortex and anterior cingulum, and concluded that this deactivation would permit the initiation of the stress response by the HPA system (Pruessner et al., 2008; Dedovic et al., 2009b).

In this study, we found an enhanced activation of the right-hippocampus and parahippocampus during the stress-task in HC but not in SZ, and left-hippocampus, parahippocampus and left-amygdala during post-stress period in HC, but not in SZ (Fig. 2). When comparing both groups we observed that similar results were headed in the same direction, showing greater activation of left-hippocampus during control-task in SZ compared with HC (Fig. 3), possibly evidencing a right deficit in this group as previously observed in relation to the theory of mind and emotional processing tasks by our group and others (de Achával et al., 2012; Andreasen et al., 2008; Rowland et al., 2009). Adding to these, when looking at the ROI analysis, we observed a sustained activation of the hippocampus and amygdala in SZ (Fig. 4), which could explain why activation is not observed when we compare different periods of the block-design paradigm.

Thus, the amygdalo-hippocampal complex seems to be involved in eliciting stress response in HC, but it was found to be dysfunctional in SZ, similarly to previous findings. In this way, it may lead to thinking in a persistent stress state, in contrast to an augmented response to a second adverse event, as proposed by the sensitization hypothesis (Glenthøj and Hemmingsen, 1997).

In accordance with this, SZ showed an inverse correlation between the performance and activation of the amygdala and hippocampus, as well as with the appraisal of frustration during debriefing, linking abnormal brain activation with behavioral symptoms. Therefore, a poor performance and high frustration might have resulted in greater activation of these limbic areas. Both amygdala and hippocampus are widely connected with frontal cortex and this could be the pathway through which high stress levels modify performance in a cognitive task. Such low performance could be responsible for the frustration perceived and mentioned by patients. Indeed, an abnormal connectivity in the prefrontal cortex-limbic thalamic nuclei-cerebellar (sensorimotor) circuit has recently been described, which could be implicated in this finding (Guo et al., 2015).

We observed activation of bilateral anterior cinguli and orbitofrontal cortex during stress task and during post-stress task (Fig. 2) in HC, but not SZ patients. Once again the results of the comparison between groups proved to be in the same direction, showing deactivation of bilateral anterior cinguli during stress (that is greater anterior cinguli activation in HC), and activation of left-orbitofrontal cortex during control-task (Fig. 3). ROI analyses showed a sustained anterior cingulum activation in patients, which could again explain a dysfunctional circuit involving prefrontal cortex. Therefore, patients with schizophrenia would have a poor control of such stress response. On the other hand, in a recent study, Guo et al. (2015) have shown an increased driving connectivity from the anterior cingulum together with medial prefrontal cortex to sensorimotor regions during resting state, as part of the prefrontal-thalamic-cerebellar circuit involved in the pathophysiology of schizophrenia (Guo et al., 2015), and our findings might extend these functional abnormalities to stress conditions. Moreover, patients with schizophrenia and their unaffected siblings shared an increased functional connectivity between medial prefrontal cortex (orbital part)

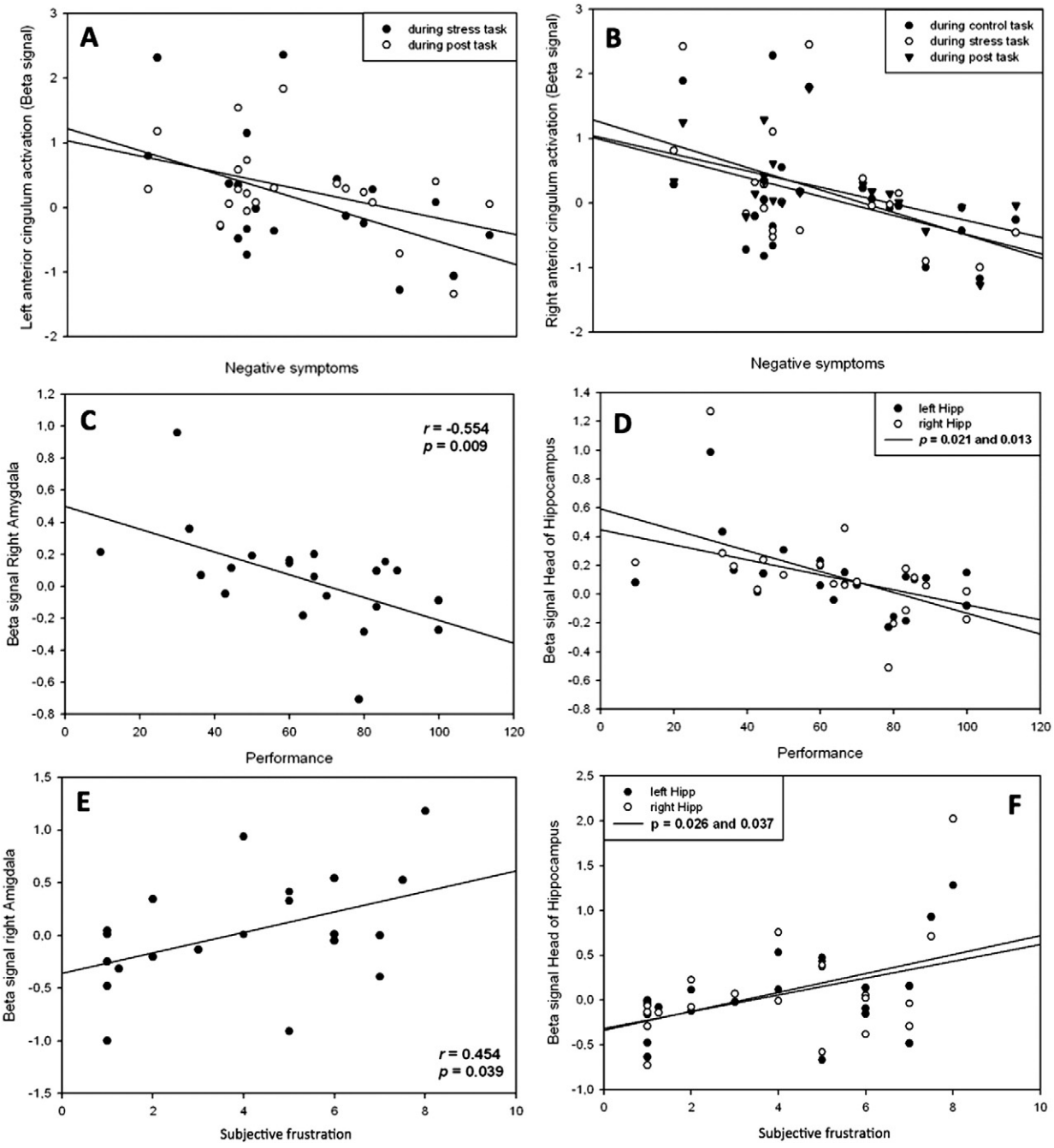

Fig. 5. Correlation between ROIs and clinical measures. Top panel: Activation in patients with schizophrenia (SZ) in A. left anterior cingulum (ACC) during stress task (STRESS > PRE contrast; $r = -0.478$, $p = 0.028$) and post task (POST > PRE contrast, $r = -0.445$, $p = 0.043$) and in B. right ACC during control task (CONTROL > PRE contrast; $r = -0.490$, $p = 0.024$) stress task ($r = -0.503$, $p = 0.020$) and post task ($r = -0.527$, $p = 0.014$ for right ACC). Middle panel: Activation during STRESS > CONTROL contrast in SZ in C. right amygdala, $r = -0.554$, $p = 0.009$ and D. left head of hippocampus ($r = -0.502$, $p = 0.021$) and right ($r = -0.535$, $p = 0.013$). Bottom panel: Activation during STRESS > PRE contrast in SZ in E. right amygdala, $r = 0.454$, $p = 0.039$ and F. bilateral head of hippocampus, $r = 0.486$, $p = 0.026$ for left and $r = 0.457$, $p = 0.037$ for right. All Pearson correlations.

and cerebellum, suggesting that this represents an endophenotype of the disease (Guo et al., in press).

The anterior cingulate cortex is involved in emotional responses (Wang et al., 2007) and decision-making processes (Critchley et al., 2000a,b). Deficits in activation of this structure in patients with schizophrenia could result not only in a lack of inhibition of stress pattern, but it could also contribute to explain their deficits in decision-making and other negative symptoms (de Achával et al., 2012, 2013; Wible et al., 2001). In fact, patients showed an inverse correlation between negative symptom severity and activation of anterior cingulum and orbitofrontal cortex, which is in agreement with the burgeoning evidence on the role of prefrontal cortex in clinical symptoms of schizophrenia (Wible et al., 2001; Ohtani et al., 2014).

The insula has been implicated in eliciting autonomic responses, as the site for the central representation of internal sensory, somatic, and endocrine states (Oppenheimer and Cechetto, 1990), and is activated in response to stress such as the TSST test (Tillfors et al., 2002). Here, insula showed bilateral activation during stress-task in HC, but not in SZ. Patients probably present a dysfunction in this area that could contribute to sensitization to stress. However, analyzing this ROI we found no differences in left-insula and even greater activity of right-insula during control compared to stress-task. This could be explained

by the fact that control-task in patients seems to be functioning as a stressful stimulus per se.

Finally, patients with schizophrenia showed a different brain activation recovery pattern characterized by activation of bilateral precuneus and left-angular gyrus. The left-angular gyrus, which presented activation during stress-task in HC, showed greater activation during post-stress task in SZ. The angular gyrus has been widely associated with calculation processes (Zamarian et al., 2009). Therefore, the protracted activity of the angular gyrus in SZ could explain a dysfunction related to the poor performance that this group showed in the present study.

The precuneus belongs to the default mode network and its deficit would lead to difficulties in self-referential and introspective processing (van Buuren et al., 2012), as well as in the deficits of social cognitive processing in siblings discordant for schizophrenia (Irani et al., 2006; de Achaval et al., 2010; Guo et al., 2014). Therefore, our results could again be associated with a social cognition processing related to the task.

The present study has a series of relevant limitations: first, we did not measure heart rate, blood pressure or cortisol as ANS or HPA variables. So we cannot determine if the stress task used here was effective to elicit a stress response. However, this paradigm was performed based on tests already proved to induce an efficient stress response.

Second, we described activation in the head of the hippocampus, whereas other studies depicted results referring to the tail of the hippocampus.

Third, all SZ were medicated with atypical antipsychotic drugs such as risperidone, olanzapine, clozapine and quetiapine. Additionally, some of the patients ($n = 6$) and one control individual were medicated with benzodiazepines. Moreover, some of them ($n = 5$) were taking Selective Serotonin Reuptake Inhibitors for depression symptoms. Since these drugs act on the limbic system, they could affect the response to the tasks.

In sum, to our knowledge this is the first study to investigate the response to stress in patients with schizophrenia using fMRI. The findings provide evidence of the involvement of limbic areas in abnormal peripheral autonomic response to stress in patients with schizophrenia, including the hippocampus, parahippocampus, amygdala, insula, anterior cingulate and orbitofrontal cortex, as previously reported. Probably, they present a pattern of persisting activation of limbic structures leading to a hypervigilance state characteristic of schizophrenia and a lack of frontal inhibition when stressors have ceased. These findings support the well known “vulnerability stress model”. Further studies are in order to understand the specific interaction between genes and environment.

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Contributors

MNC, MFV, KJB, CBN and SMG developed the research project, MNC and MFV developed the fMRI testing paradigm, MNC, MFV, NB, EP, LJD, MGG, DdA, and JP obtained clinical and research data, MNC, MFV, CBN, KJB and SMG analyzed and interpreted the data, MNC and SMG wrote the first draft of the manuscript, and all authors contributed to the final manuscript.

Conflict of interest

Charles B. Nemeroff, M.D., Ph.D. Declaration of Financial/Propriety Interest 2011–2014. Research/Grants: National Institutes of Health (NIH). Consulting: Xhale, Takeda, SK Pharma, Shire, Roche, Lilly, Allergan, Mitsubishi Tanabe Pharma Development America, Taisho Pharmaceutical Inc., Lundbeck, Prismic Pharmaceutical. Stockholder: CeNeRxBioPharma, PharmaNeuroBoost, Revaax Pharma, Xhale, Celgene, Seattle Genetics, Abbvie. Scientific Advisory Boards: American Foundation for Suicide Prevention (AFSP), CeNeRxBioPharma (2012), National Alliance for Research on Schizophrenia and Depression (NARSAD), Xhale, PharmaNeuroBoost (2012), Anxiety Disorders Association of America (ADAA), Skyland Trail. Board of Directors: AFSP, NovaDel (2011), Skyland Trail, Gratitude America, ADAA. Income sources or equity of $10,000 or more: PharmaNeuroBoost, CeNeRxBioPharma, NovaDel Pharma, ReevaxPharma, American Psychiatric Publishing, Xhale. Patents: Method and devices for transdermal delivery of lithium (US 6,375,990B1). Method of assessing antidepressant drug therapy via transport inhibition of monoamine neurotransmitters by ex vivo assay (US 7,148,027B2). Speakers Bureau: None. Honoraria: Various. Royalties: Various. Expert Witness: Various.

All of the other authors declare no potential conflict of interests.

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