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Brain activation induced by psychological stress in nonpsychotic siblings of patients with schizophrenia

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ABSTRACT

There is compelling evidence that environmental factors together with a large predisposing genetic component contribute to the risk for schizophrenia. Among such factors, psychosocial stress has been considered of paramount importance prior to the onset of psychotic symptoms. In order to characterize the brain response to mental arithmetic stress in individuals genetically predisposed to schizophrenia, we employed 3T-fMRI in 13 nonpsychotic siblings of patients with schizophrenia and in 13 healthy individuals. After a period of 6 min of resting state acquisition, a block design was utilized, including three blocks of a 1-min control-task, 1-min stress-task and 1-min rest after task. Nonpsychotic siblings displayed several differences in brain activity as compared with healthy individuals, including failure to engage the right hippocampus and orbitofrontal cortex (OFC) during stress and shortly thereafter. In addition, in this group hippocampal function was associated with cognitive performance rather than perceived stress. Indeed perceived stress was in contrast associated with activation of bilateral OFC and insulae. The pattern of brain activation observed may represent the CNS correlate of previous observations on heightened sensitivity to psychosocial stress in persons at increased genetic risk for schizophrenia. Its potential usefulness as a marker of increased genetic predisposition to schizophrenia requires further investigation.

Introduction

In most cases of schizophrenia, heritability seems to be determined by an as-yet uncertain, and probably heterogeneous, combination of unfavorable genetic variants, usually in the form of single nucleotide polymorphisms (SNPs, [36]). The individual contribution of each vulnerability SNP to the expression of the complex behavioral phenotype of schizophrenia is very small, and therefore there is general agreement that two factors are necessary to significantly increase the likelihood to develop this disorder: coexistence of a sufficient number of predisposing SNPs, and one or more adverse environmental factors. Among the latter, it is thought that some insults occur at early (e.g., prenatal or perinatal) critical neurodevelopmental periods, as well as other factors operating closer to the disease onset. This is the basis for the stress-diathesis model of schizophrenia, formulated three decades ago [30]. Psychosocial stress combined with early adverse life events have been consistently considered of paramount importance among factors immediately preceding symptom onset [3,22]. How exactly stress interacts with genetic risk factors to increase risk for schizophrenia remains, however, obscure at this time. Endophenotypes, measurable components unseen by the unaided eye along the pathway between disease phenotype and distal genotype, have emerged as an important concept in the study of complex neuropsychiatric diseases. An endophenotype may be neurophysiological, biochemical, endocrinological, neuroanatomical, cognitive, or neuropsychological in nature and represent simpler clues to genetic

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underpinnings than the disease syndrome itself, promoting the view that psychiatric diagnoses can be decomposed or deconstructed, which can result in more straightforward—and successful—genetic analysis. Thus, the endophenotype should be associated with the illness in the population, should be heritable, primarily state-independent (manifests in an individual whether or not illness is active), co-segregate with illness within families, and should be found in affected and nonaffected family members at higher rate than in the general population [17]. Therefore, the identification of endophenotyoes or intermediate phenotypes [20] might be useful in defining precise pathophysiological pathways beginning with abnormal expression of genes and ending in expression of disease symptoms. Brain activity endophenotypes have been considered potentially useful in this search [20] because they likely represent underlying disease mechanisms, midway between altered genes and clinical symptoms.

In the present study, we sought to define brain areas involved in responses to psychosocial stress in nonpsychotic siblings of patients with schizophrenia, who share with them a substantial number of the gene variants presumably conferring risk. We specifically hypothesized that, in agreement with previous data of emotional [29] and peripheral autonomic [3] responses to stress in siblings discordant for schizophrenia, nonpsychotic siblings of patients would display heightened intensity and prolonged duration of activation of limbic brain areas that mediate stress regulation. We hypothesized abnormal activity would likely involve the hippocampal formation and amygdala [44,6,34], and orbitofrontal cortex, anterior insula, and anterior cingulum (e.g., [27]). We further predicted an abnormal lateralization of such responses, such that deficits would preferably involve right-hemisphere structures, as previously observed in a variety of paradigms by our group and others [26,10,42]. In the image analyses, we segmented the hippocampal formation, given the compelling evidence of a differential involvement of diverse sectors in schizophrenia (see Ref. [40] for a review).

Methods

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

Siblings (Sb)

Thirteen siblings of patients with schizophrenia (7 females, aged 25 ± 6 years) were recruited from ambulatory patients seen at the Psychiatry Service, and were aged 18–50 years. They were enrolled consecutively and exclusion criteria included (a) the lifetime presence of any DSM-IV-TR [1] Axis I psychotic disorder diagnosis as confirmed with a Composite International Diagnostic Interview [35] administered by a consultant psychiatrist (SMG or MNC), and (b) a medication history of antipsychotics or mood stabilizers.

Healthy controls (HC)

Thirteen healthy volunteers (7 females, aged 25 ± 4 years, range years) were recruited from the local community. Exclusion criteria included (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.

Procedures

Screening tests

All participants were screened for premorbid intelligence with the Word Accentuation Test (WAT; [12,10] and for depressive symptoms with the Hamilton depression test (HAM-D; [19].

fMRI stimuli

We used a stress paradigm based on previous studies [13,11], which consisted in a period of 6 min of resting state (PRE) acquisition followed by a block design which had three blocks of 1-min CONTROL-task, 1 min STRESS-task and 1 min rest after task (POST). CONTROL-task consisted in a one-digit sum of three terms, which had a very low difficulty level. 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 also evaluated 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 the scan and during the stress task, the level of effort, task difficulty and frustration generated by the stress task (on a Likert scale of 1 to 10; adapted from Ref. [43].

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.

Statistical analysis

Analysis of demographical data

Discrete variables in siblings and controls were compared using a chi-square test. Continuous variables were compared with an independent-samples *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 SPSS v18.0 (SPSS Inc.).

fMRI analysis

Imaging processing. Image processing was carried out using SPM8 (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 and spatially

normalized to the stereotactic space of Talairach and Tournoux [39] using Montreal Neurological Institute reference brain [2]. The volumes were spatially smoothed by an isotropic Gaussian kernel of 8 mm at full width half-maximum [15].

Image statistical analysis. Statistical analysis was carried out using SPM8 (Wellcome Department of Cognitive Neurology, London, UK) implemented in MATLAB (Mathworks Inc., Sherborn, MA, USA). 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. Linear contrasts: STRESS > CONTROL, POST > CONTROL, CONTROL > PRE, STRESS > PRE and POST > PRE were evaluated for each subject.

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

We used FWE corrected $p < 0.05$.

ROI analysis. We explored activation during different contrasts in specific regions based upon prior hypothesis, namely a) previous reported areas related to stress response [9,11,24] and to cognitive task [21], b) our previous studies [4,3], and c) the results of the experimental CONDITION effect. These ROIs were defined from automated anatomical labeling (AAL) atlas. We thus studied hippocampus, amygdala, cingulate gyrus, and insula. For the hippocampus we performed a division for head, body and tail using a validated protocol developed by Pruessner et al. [33].

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

Subsequently, 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 the mean beta values of all ROIs between groups and repeated measures ANOVA to compare each experimental condition within group followed by Bonferroni *post hoc* test. The brain activity was reported in the MNI system. We assumed $p < 0.05$ for all ROIs results.

Finally, we made a Pearson correlation analysis between beta signal and clinical measures. We used two-tailed $p < 0.05$.

Results

Table 1 presents the demographic and clinical characteristics of the study participants. Both groups were similar regarding age, gender, years of education and premorbid intelligence. One sibling was treated with an SSRI. Two siblings were left-handed. Siblings had a poorer performance in the arithmetics tasks and the perceived stress reported was similar in both groups. (Table 1).

ANOVA results

GROUP influenced brain activation at bilateral superior frontal and precentral gyri, left postcentral gyrus, left superior and inferior parietal gyrus and left superior occipital gyrus ($F = 15.02$; FWE corrected $p < 0.05$).

CONDITION affected several brain areas, some bilaterally and some in one hemisphere. Anterior cingulate cortex, orbitofrontal cortex, superior and middle frontal gyri, precentral gyri, superior and inferior parietal gyri, angular gyri, lingual gyri, fusiform gyri, occipital area, cerebellar hemispheres, and cerebellar vermis were all affected bilaterally. CONDITION affected activity in left hippocampus, parahippocampus, amygdala, inferior frontal gyrus, postcentral gyrus, precuneus and inferior temporal gyrus. Supplementary motor area was the only right hemisphere structure significantly affected by CONDITION ($F = 11.44$; FWE corrected $p < 0.05$).

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

Within-group comparisons

In healthy individuals, STRESS > CONTROL task activation contrasts revealed higher activation in bilateral superior and middle frontal gyri, bilateral precentral area and left inferior frontal gyrus (Fig. 1A). Nonpsychotic siblings displayed activation of left inferior parietal gyrus, angular gyrus, precentral area and right superior frontal gyrus during the same contrast (Fig. 1B).

During POST > CONTROL contrast healthy individuals displayed activation of bilateral anterior cingulum and right orbitofrontal cortex (Fig. 1C). However, siblings did not show, during the same contrast, activation of any brain area (Fig. 1D).

Siblings vs. healthy subjects comparisons

Whole brain analysis showed greater activation of left precuneus during post period in siblings compared with control individuals (POST > PRE contrast; FWE corrected $p = 0.049$ across a small volume of interest).

ROIs analyses

In healthy individuals, we observed higher activation during stress than control tasks in both left and right anterior cinguli (Fig. 2A). However, siblings of patients with schizophrenia did not present significant within-group differences in this area (Fig. 2A).

Healthy subjects presented higher activation during stress compared with control task at the right orbitofrontal cortex, as well as lower activation than post period. Siblings did not present significant within-group differences in these analyses (Fig. 2B).

In healthy controls, the body of the right hippocampus had higher activation during stress compared with control task and post period. However, siblings did not present significant within-group differences in these analyses (Fig. 2C). In contrast, healthy controls displayed greater activation in both left and right tail of the hippocampus during stress compared with control and post-task. Again, siblings did not present significant differences (Fig. 2D).

Correlation analyses

Siblings of patients with schizophrenia showed a positive correlation between performance and body of hippocampus during the control task (Fig. 3A), as well as tail of hippocampus during control and stress task (Fig. 3B).

In contrast, healthy controls presented a positive correlation between perceived stress scale score and left amygdala activity (Fig. 4A) and right body of hippocampus during stress (Fig. 4B).

However, siblings of patients with schizophrenia did not exhibit correlations with these brain areas, but a positive correlation between perceived stress score and bilateral orbitofrontal cortex during stress (Fig. 5A), as well as with bilateral insula during stress task (Fig. 5B).

Discussion

The main finding of this study is that nonpsychotic siblings of patients with schizophrenia resemble healthy persons regarding

Table 1
Demographic characteristics.

	Siblings (n = 13)	Controls (n = 13)	Statistic	p
Age (years)	25 ± 6	25 ± 4	t = 0.113	0.911
Female, n (%)	7 (53.8)	7 (53.8)	X^2 < 0.001	1.000
Education (years)	14 ± 2	15 ± 2	t = −1.432	0.165
Smokers, n (%)	3 (23.1)	2 (15.4)	X^2 = 0.248	0.619
HAM-D[a]	3 ± 3	2 ± 2	X^2 = 0.908	0.373
WAT[b]	30 ± 3	32 ± 5	t = −1.513	0.143
Performance (% correct responses in mental arithmetic task)	36 ± 22	57 ± 18	t = −2.611	0.015
PSS[c], n (%)	27 ± 8	24 ± 10	t = −0.630	0.535
SSRI[d], n (%)	1 (7.7)	–	–	–

All values are showed as n (%) or mean ± SD.
[a] HAM-D: Hamilton depression score.
[b] WAT: Word Accentuation Test.
[c] PSS: Perceived Stress Scale.
[d] SSRI: Selective Serotonin Reuptake Inhibitor.

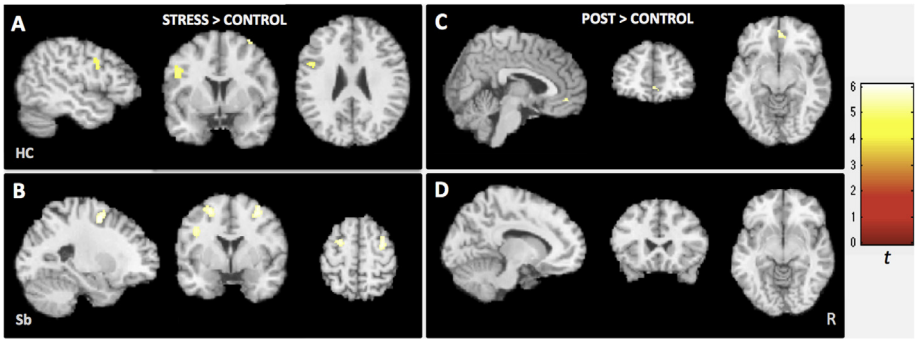

Fig. 1. Brain activation during mental stress (STRESS > CONTROL contrast) in (A) healthy subjects (x = −53, y = 2, z = 31) and (B) siblings discordant for schizophrenia (x = −28, y = −2, z = 64); and during POST > CONTROL contrast in (C) healthy subjects (x = 0, y = 44, z = −4), and (D) siblings (x = −15, y = 21, z = −2). ANOVA analysis within group. All results FWE corrected p = 0.05.

activation of dorsolateral prefrontal cortex areas during mental arithmetic tasks, but display a different pattern of limbic structure activity during stress, in the form of 1) failure to engage the right hippocampus and orbitofrontal cortex (OFC) during stress and shortly thereafter, in comparison with rest and a non-stressful mental task, 2) evidence of participation of hippocampal function in the performance of mental arithmetic tasks but not in response to stress, as was observed in healthy individuals, 3) correlation between activation of bilateral OFC and insulae with perceived stress induced by mental arithmetic, suggesting a more robust limbic activation in relation to mental stress, and 4) lack of a relationship between left amygdala activation and stress perception, as was evident in the present group of healthy individuals.

In the present study we have focused on the involvement of hippocampal formation in psychological stress related to mental arithmetic given the burgeoning evidence of a role of this structure in schizophrenia [38,28,46]. In fact, we previously found a pattern of persisting activation of hippocampus together with other limbic areas in response to stress in patients with schizophrenia [5]. Specifically, activation in these limbic areas are triggered by simple tasks and persist during and after stress. This might represent a neurobiological signature of hyperreactivity to normal stressful situations and hypervigilance state characteristic of this disease [5]. In the present study, we observed failure to engage the hippocampus during stress and shortly thereafter, in comparison with rest and a non-stressful mental task. And when we analyzed the hippocampus as a region of interest in this group, it showed a flattened activation throughout testing. However, in siblings, the absence of significant variations in brain activity during control task vs. stress task vs. post-stress epochs is difficult to interpret. One possibility is that stressful limbic responses could begin in the control task, and then continue during the post-stress phase, thus obscuring any significant difference in brain activation across all three experimental settings. This view is supported by previous data pointing to protracted autonomic activity in a similar paradigm and sample of individuals [3]. Also, it could be in accordance with previous studies carried out on patients with schizophrenia in both peripheral and central nervous system measures in response to stress [4,5]. In such case, similarities in siblings discordant for schizophrenia suggest a heritable trait of abnormal limbic functioning possibly representing an endophenotype. Thus, an abnormal functioning of limbic system expressed as hypervigilance state and hyperreactivity to stress in individuals who have already developed the disease could be a result of a progression from initial state in which limbic areas are subtly affected.

The psychological stress test is a mental arithmetic test with a strong cognitive attentional/working memory component [38,28,46]. A series of task-associated hippocampal activation abnormalities have been described in schizophrenia, especially during declarative memory tasks evoking conjunctive memory phenomena (e.g., [45,32]). Most interesting, there is a consistent alteration in schizophrenia in the connectivity between prefrontal cortex and medial temporal lobe areas [14,25], where activation was elicited by a mental arithmetic task in the present study.

Whereas in healthy individuals activity in the hippocampi was related to the severity of subjective stress perception, and not to mental performance, the opposite was true for individuals at heightened genetic risk of schizophrenia. A possible explanation is that nonpsychotic siblings of patients with schizophrenia represent a heterogeneous group with variable genetic predisposition affecting hippocampal function, hence the relationship between hippocampal activation and performance. Thus, whereas some individuals presumably displayed normal hippocampal involvement in the task, related in turn with better cognitive performance,

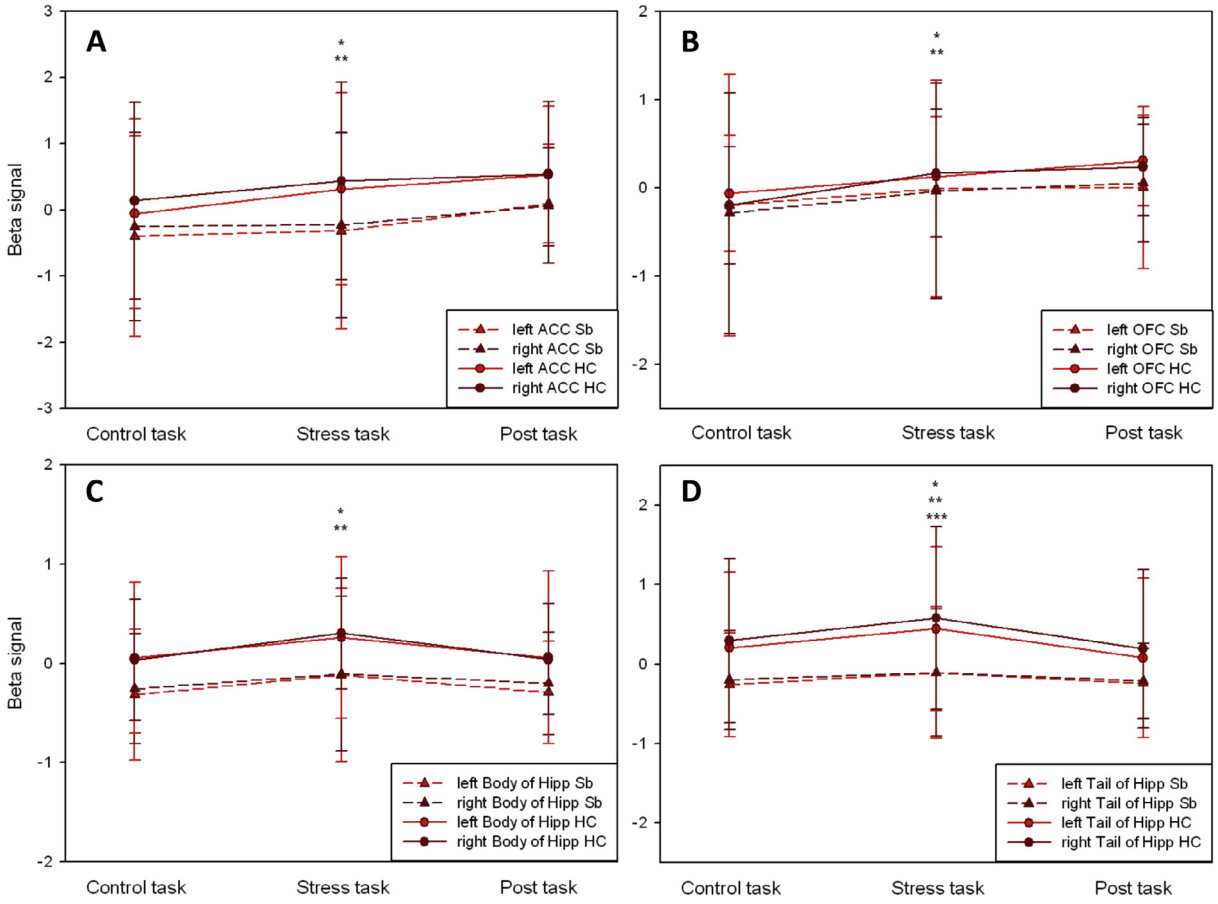

Fig. 2. Activation of ROIs in both groups. (A) Activation of anterior cingulum (ACC) during all conditions in siblings (Sb) and healthy controls (HC). $^{*}p = 0.003$ vs. control task for left and $^{**}p = 0.002$ vs. control task for right ACC in HC group; [(STRESS > PRE) > (CONTROL > PRE)] contrast; (B) Orbitofrontal cortex (OFC) during all conditions in Sb and HC. $^{*}p = 0.002$ vs. control [(STRESS > PRE) > (CONTROL > PRE)], and $^{**}p = 0.012$ vs. post period [(STRESS > PRE) > (POST > PRE)] for right OFC in HC; (C) Body of hippocampus (Body Hipp) during all conditions. $^{*}p = 0.018$ vs. control task [(STRESS > PRE) > (CONTROL > PRE)], and $^{**}p = 0.036$ vs. post task [(STRESS > PRE) > (POST > PRE)] for right Body Hipp in HC; (D) Tail of hippocampus (Tail Hipp) during all conditions. $^{*}p = 0.009$ vs. control task [(STRESS > PRE) > (CONTROL > PRE)] for left and right Tail Hipp, and $^{**}p = 0.015$ for left and $^{***}p = 0.012$ for right Body Hipp vs. post task for HC [(STRESS > PRE) > (POST > PRE)] contrast. All repeated measures ANOVA followed by Bonferroni *post hoc* test.

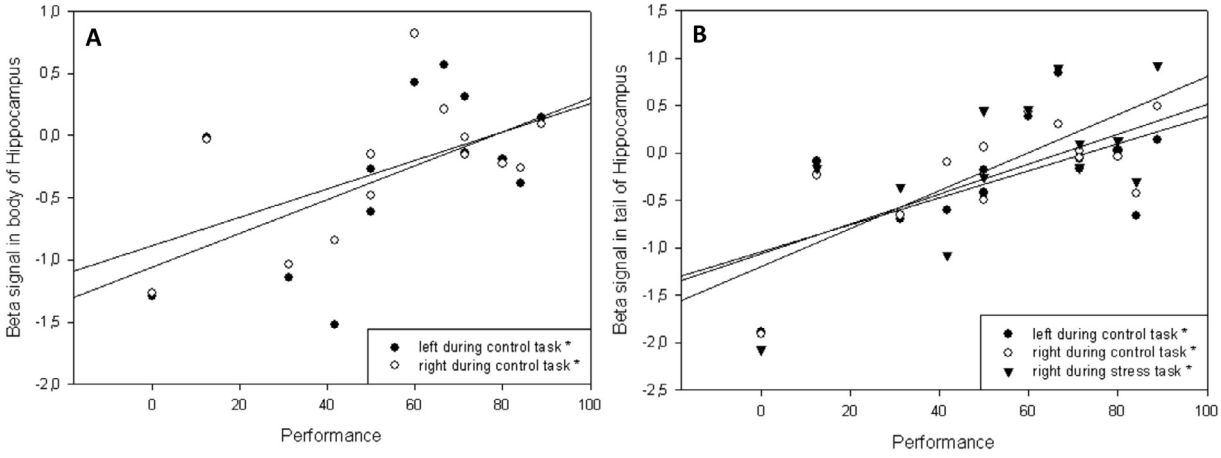

Fig. 3. Correlation between ROIs and performance. Activation in siblings (Sb) in (A) body of hippocampus (Hipp) during control task (CONTROL > PRE contrast; $^{*}r = 0.562$, $p = 0.046$ for left and $^{*}r = 0.565$, $p = 0.044$ for right), and in (B) tail of Hipp during control task ($^{*}r = 0.597$, $p = 0.031$ for left and $^{*}r = 0.697$ $p = 0.008$ for right) and during stress task (STRESS > PRE contrast; $^{*}r = 0.702$, $p = 0.010$ for right).

others might have disease-related abnormalities in hippocampal function, which was revealed in the group analysis as a correlation between performance and hippocampal activation. Such a correlation was absent in healthy persons, who displayed significantly better mental arithmetic abilities as a group. Remarkably, the relationship between mental arithmetic performance and hippocampal activation was most robust during the control mental arithmetic task, involving not only the hippocampal tail (presumably reflecting activation of the output region of the hippocampal formation) but also the body of the hippocampus, where the

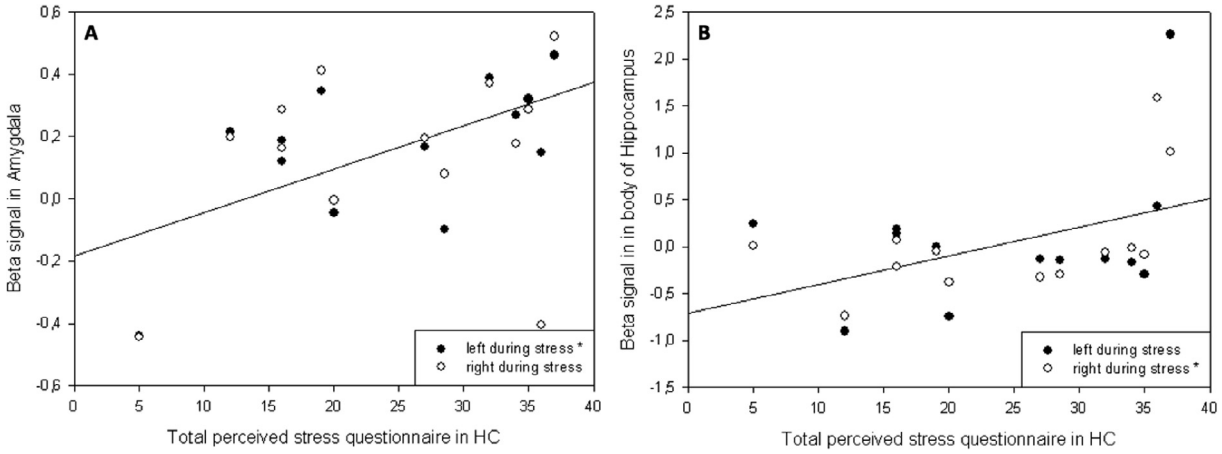

Fig. 4. Correlation in healthy controls (HC) between total perceived stress questionnaire and activation in (A) Amygdala ($^{*}r = 0.605$, $p = 0.029$ for left during STRESS > CONTROL contrast), and in (B) body of Hippocampus ($^{*}r = 0.594$, $p = 0.032$ for right during STRESS > PRE contrast).

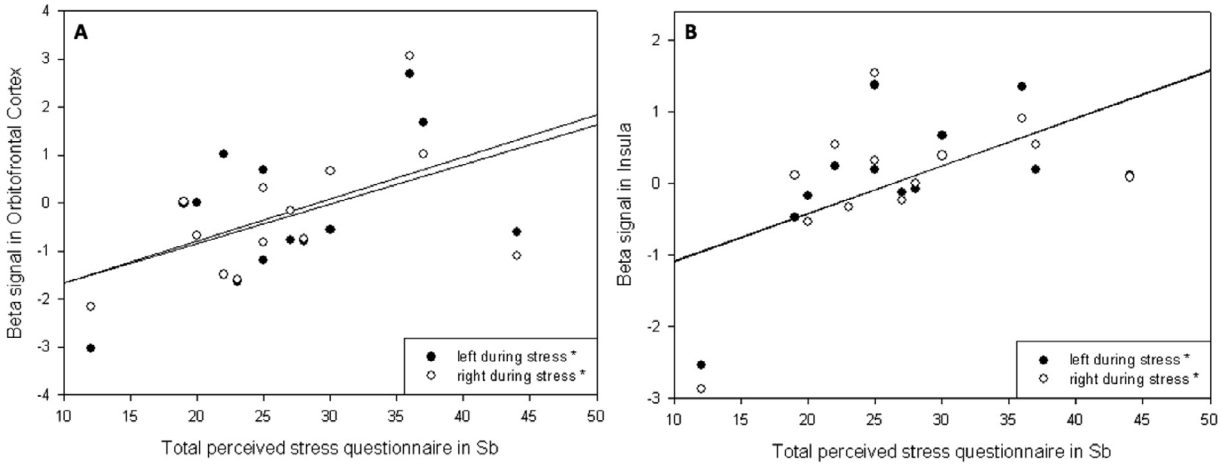

Fig. 5. Correlation in non psychotic siblings (Sb) between total perceived stress questionnaire and activation in (A) Orbitofrontal Cortex ($^{*}r = 0.607$, $p = 0.028$ for left; $^{*}r = 0.568$, $p = 0.043$ for right during STRESS > PRE contrast), and in (B) Insula ($^{*}r = 0.570$, $p = 0.042$ for left; $^{*}r = 0.561$, $p = 0.046$ for right during STRESS > PRE contrast).

trisynaptic, mostly unidirectional processing in this area is known to occur [31].

On the other hand, despite perceived stress was similar in both controls and siblings groups, perception of stress severity in siblings was robustly associated with activation of structures known to mediate autonomic/visceral responses to negative emotions in normal conditions, namely insulae and medial orbitofrontal cortex. This might represent the central correlate of a higher sensitivity to stress in persons with increased genetic risk for schizophrenia, as previously demonstrated for psychological appraisal of aversive stimuli [29] and for peripheral autonomic correlates of stress [4,3]. Indeed, the lack of differences in perception of stress in siblings compared with healthy controls could be an expression of affective flattening characteristic of schizophrenia and also described in non-affected siblings [7,37], or even it could be a sign of alexithymia as part of their social dysfunction [7].

Finally, we found lack of a relationship between left amygdala activation and stress perception, as was evident in the present group of healthy individuals, in accordance with previous data suggesting that left amygdala activity depends on salience of psychosocial stimuli [8]. Thus, our prediction of lack of dominant activation of right limbic structures during stress was confirmed in this study, in accordance with previous findings by our group and others in siblings discordant for schizophrenia challenged with social cognitive tasks [10,16,42,18].

The present study had some methodological limitations that should be taken into account when interpreting the results. First, the relatively small sample size might have obscured significant relationships between patterns of brain activity and psychological stress. Thus, the present study should be considered preliminary and its findings confirmed in larger samples. Second, all participants were recruited at the same center and were homogeneous in culture, ethnicity, and geographical distribution, thus limiting the generalizability of results to all individuals at heightened genetic risk of schizophrenia. Third, there are studies that observed a familiar co-aggregation between schizophrenia, bipolar disorder and schizoaffective disorder [23,41], suggesting that depression diagnosis could contribute to the pattern of limbic system activation that we found here. However, only one sibling was taking an antidepressant and this subject had a low score of Hamilton depression test at time of admission for this study. Anyway, the fact that one sibling was taking an antidepressant might have contributed to the observed results regarding performance and brain activation during fMRI scanning. Fourth, being cross-sectional, the present results do not permit to infer causation between patterns of brain activation and poor performance during paradigm or its correlations with subjective stress. Finally, the presence of two non right-handed siblings in the present sample might have introduced anatomic variability in brain activation [8], thus rendering the present sample unlikely to permit detection of intra-group variability in relation to stress.

Nonetheless, the present results add to accumulating evidence of limbic abnormalities in siblings discordant for schizophrenia probably reflecting abnormal functioning of right-hemisphere structures. The precise heritability of the brain functional abnormalities described herein, as well as their influence in susceptibility to develop psychotic symptoms vis à vis adverse psychosocial stimuli, necessitate further investigation, especially in prospective studies with at-risk subjects.

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