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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 = $3.75 \times 3.75 \times 4$). 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 normal-

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