Stress and Inflammation Reduce Brain-Derived Neurotrophic Factor Expression in First-Episode Psychosis: A Pathway to Smaller Hippocampal Volume

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ABSTRACT

Background: Reduced brain-derived neurotrophic factor (BDNF) levels have been reported in the serum and plasma of patients with psychosis. The aim of this cross-sectional case-control study was to investigate potential causes and consequences of reduced BDNF expression in these patients by examining the association between BDNF levels and measures of stress, inflammation, and hippocampal volume in first-episode psychosis.

Method: Brain-derived neurotrophic factor, interleukin (IL)-6, and tumor necrosis factor (TNF)-α messenger RNA levels were measured in the leukocytes of 49 first-episode psychosis patients (*DSM-IV* criteria) and 30 healthy controls, all aged 18 to 65 years, recruited between January 2006 and December 2008. Patients were recruited from inpatient and outpatient units of the South London and Maudsley National Health Service Foundation Trust in London, United Kingdom, and the healthy controls were recruited from the same catchment area via advertisement and volunteer databases. In these same subjects, we measured salivary cortisol levels and collected information about psychosocial stressors (number of childhood traumas, number of recent stressors, and perceived stress). Finally, hippocampal volume was measured using brain magnetic resonance imaging in a subsample of 19 patients.

Results: Patients had reduced BDNF (effect size, $d = 1.3$; $P < .001$) and increased IL-6 (effect size, $d = 1.1$; $P < .001$) and TNF-α (effect size, $d = 1.7$; $P < .001$) gene expression levels when compared with controls, as well as higher levels of psychosocial stressors. A linear regression analysis in patients showed that a history of childhood trauma and high levels of recent stressors predicted lower BDNF expression through an inflammation-mediated pathway (adjusted $R^2 = 0.23$, $P = .009$). In turn, lower BDNF expression, increased IL-6 expression, and increased cortisol levels all significantly and independently predicted a smaller left hippocampal volume (adjusted $R^2 = 0.71$, $P < .001$).

Conclusions: Biological changes activated by stress represent a significant factor influencing brain structure and function in first-episode psychosis through an effect on BDNF.

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Several studies have reported reduced brain-derived neurotrophic factor (BDNF) levels in the brain[1] and in the serum and plasma of patients with schizophrenia.[2–7] Brain-derived neurotrophic factor is widely expressed in the adult mammalian brain and is known to play a crucial role promoting proliferation, regeneration, and survival of neurons.[8] This fact is particularly important in this context, since a number of studies have shown brain volume changes at the onset of psychosis or during the transition to psychosis, suggesting a critical role for neuroplasticity, especially in the hippocampus, in the development of psychosis.[9–11] Moreover, BDNF has also been involved in more complex processes in adulthood, such as regulation of cognitive function,[12] which is known to be impaired in patients with psychosis.[13,14] However, the clinical and biological mechanisms behind the lower BDNF levels and the progressive brain volume changes in psychosis are still unknown.

Stress and the biological systems involved in the stress response have been suggested to play a role in BDNF changes. Indeed, animal models have shown that chronic stress down-regulates hippocampal BDNF messenger RNA (mRNA) expression and impairs processes of neuronal branching and neurogenesis.[15] High levels of both glucocorticoid hormones and proinflammatory cytokines, 2 key players in the response to stress, have been associated with decreased BDNF levels[16,17] and with changes in hippocampal neuronal function such as dendrite atrophy, neuronal death, and reduced neurogenesis.[18–21] Interestingly, we and others have shown both abnormal glucocorticoid function and increased inflammatory markers in patients with first-episode psychosis.[22,23] Furthermore, we recently showed a negative correlation between cortisol levels and left hippocampal volume in first-episode psychosis.[24] Previous studies have also shown that childhood trauma, a powerful psychosocial stressor, is associated with smaller left hippocampal volume in patients with other psychiatric disorders such as depression and posttraumatic stress disorder.[25,26] However, the potential pathways linking stressful events to BDNF and to hippocampal volume have not been studied in psychosis.

In the present study, our aim was to investigate the pathways leading from psychosocial stress to reduced BDNF levels in patients with first-episode psychosis and to evaluate the putative consequence of these reduced BDNF levels on hippocampal volumes. Of relevance is that BDNF in the blood seems to reflect the BDNF content in the brain; this notion is substantiated by animal

experiments, showing that serum BDNF levels are correlated with BDNF expression in cortical and hippocampal brain regions,[27–29] and further supported by a positive correlation between plasma and cerebrospinal fluid BDNF levels recently reported in drug-naive first-episode psychosis patients.[30] We have specifically measured BDNF expression in leukocytes, as the leukocyte gene expression profile shows similarities to those observed in the brain, especially for genes linked to neurotransmitters, cytokines, hormones, and growth factors.[29,31] Brain-derived neurotrophic factor mRNA expression has been recently found to be decreased in leukocytes of depressed patients[32]; however, this topic has never been studied in psychosis. Moreover, we have measured (1) psychosocial stress (childhood trauma, recent stressful events, and perceived stress), (2) inflammatory markers (leukocyte mRNA levels of interleukin [IL]-6 and tumor necrosis factor [TNF]-α), (3) diurnal salivary cortisol levels, and (4) hippocampal volume (via magnetic resonance imaging [MRI]). Some of the subjects described in this article belong to a group of patients and controls previously described.[22,24] This article is now advancing this work, presenting for the first time the data on BDNF gene expression and on inflammatory markers in these patients and the association between these variables and the stress and imaging measures.

- Biological abnormalities triggered by stress contribute to brain structure abnormalities at the onset of psychosis.
- Targeting biological pathways involved in the stress response might help to prevent development of psychosis and improve clinical outcome of patients with psychosis.

Clinical Points

METHOD

Subjects

First-episode psychosis patients were recruited in London, United Kingdom, from inpatient and outpatient units, part of the South London and Maudsley National Health Service (NHS) Foundation Trust in South-East London, from January 2006 to December 2008. The recruitment strategy was based on contacting inpatient and outpatient services, interviewing staff, reviewing clinical notes, and approaching all subjects aged 18 to 65 years who presented for the first time to these services for a functional psychotic illness (*ICD-10* F10–F19, excluding code F1X.0 for acute intoxication; F20–F29; and F30–F39 psychotic codings).[33] Patients with organic psychosis or learning disabilities or who were not fluent in English were excluded from the study. Controls were recruited from the same catchment area as the patients through advertisement in local newspapers, hospitals, and job centers, as well as from existing volunteer databases. Controls were screened using the Psychosis Screening Questionnaire[34] and were excluded if they met criteria for a present or past psychotic disorder. This cross-sectional case-control study was approved by the local Research Ethics Committee in accordance with the code of ethics of the World Medical Association, and written informed consent was obtained from all participants.

We recruited and assessed 49 patients with first-episode psychosis and 30 healthy controls. All subjects had an assessment of BDNF, IL-6, and TNF-α expression; only 19 of these patients agreed to undergo an MRI scan. Twenty-seven patients received a *DSM-IV* diagnosis of schizophrenia/schizophreniform disorder; 15, of schizoaffective or affective psychosis; and 7, of psychotic disorder not otherwise specified. Ten patients were drug naive, 36 were on treatment with an atypical antipsychotic (mostly olanzapine, n = 19), and 3 were on treatment with a typical antipsychotic. The mean (SE) duration of antipsychotic treatment was 33.4 ± 5.8 days (range, 0–170 days).

Questionnaires and Clinical Assessment

Validation of clinical diagnosis was obtained using the Operational Criteria,[35] reviewing the case notes in the first month following first contact with services. We collected information about stressful life events that occurred in the previous 6 months using a brief life events questionnaire,[36] and we measured perceived stress in the previous month using the Perceived Stress Scale.[37] Information about childhood trauma was also collected using a modified version of the Childhood Experience of Care and Abuse Questionnaire.[38] Please see supplementary material at PSYCHIATRIST.COM for details on childhood trauma assessment.

Gene Expression Analyses

Blood samples for gene expression of BDNF, IL-6, and TNF-α were collected using PAXgene Tubes (Qiagen SpA; Milan, Italy). The time of blood collection varied for each subject, and subjects did not fast before collection. After blood samples were withdrawn, PAXgene Tubes were kept for 2 hours at room temperature and then stored at −80°C until they were processed. Isolation of RNA was performed using the PAXgene Blood RNA Kit (Qiagen SpA; Milan, Italy) according to manufacturer's protocols. Please see online supplementary material for details on gene expression analyses.

Salivary Cortisol Assessment

Saliva samples were collected to measure salivary cortisol using Salivettes (Sarstedt; Leicester, United Kingdom). Methods of sample collection and analyses have been described in detail before.[22] Subjects were instructed to collect saliva samples immediately after awakening and again at 12:00 PM and at 8:00 PM. Cortisol levels during the day were measured as area under the curve (AUC) of cortisol levels at the 3 timepoints, following the calculation of the AUC derived from the trapezoid formula.[39]

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Table 1. Sociodemographic Characteristics, Stress Variables, and Interleukin (IL)-6, Tumor Necrosis Factor (TNF)-α, and Brain-Derived Neurotrophic Factor (BDNF) Gene Expression in First-Episode Psychosis Patients and Healthy Controls

Variable	Patients (N = 49)	Controls (N = 30)	Statistical Test	*df*	*P* Value[a]
Age, mean ± SE, y	28.2 ± 0.9	27.0 ± 0.8	$t = -1.0$	1,77	.3
Sex, male/female, n/n	33/16	19/11	$\chi^2 = 0.1$		.8
Sex, male, %	67.3	63.3			
Body mass index,[b] mean ± SE	24.8 ± 0.8	23.5 ± 0.8	$t = -1.2$	1,60	.3
Race/ethnicity, white/other, n/n	11/38	9/21	$\chi^2 = 0.6$		.6
Race/ethnicity, white British, %	22.4	30.0			
No. of recent stressors, mean ± SE	2.3 ± 0.2	1.3 ± 0.2	$t = -3.0$	1,71	**.004**
Perceived Stress Scale score, mean ± SE	21.4 ± 1.1	12.0 ± 1.2	$t = -5.6$	1,71	**<.001**
Childhood trauma, no/yes, n/n	9/32	17/11	$\chi^2 = 10.7$		**.002**
Percentage with at least 1 trauma	78.0	39.3			
Day cortisol, nmol × h/L, mean ± SE	79.2 ± 4.6	77.6 ± 7.7	$t = -0.2$	1,43	.9
IL-6 gene expression, mean ± SE	1.67 ± 0.10	1.04 ± 0.09	$t = -4.6$	1,73	**<.001**
TNF-α gene expression, mean ± SE	1.66 ± 0.06	1.04 ± 0.05	$t = -7.7$	1,75	**<.001**
BDNF gene expression, mean ± SE	0.54 ± 0.05	1.17 ± 0.10	$t = 5.5$	1,77	**<.001**

[a]Boldface type indicates significance.
[b]Body mass index calculated as kg/m^2.

Hippocampal Volume

Magnetic resonance imaging scans were acquired on 19 of the recruited patients with a GE Signa 1.5-T system (GE Medical Systems; Milwaukee, Wisconsin) at the Maudsley Hospital, London, United Kingdom. Please see online supplementary material for details on MRI scans acquisition. Hippocampal volume was measured blind to group status or BDNF, cytokine, and cortisol levels by a single rater using the software program Measure (version 0.8; Johns Hopkins University; Baltimore, Maryland). This image analysis program uses stereologically unbiased estimation of volume. The program and the measurement procedure have been described in detail elsewhere.[40]

Data Analyses

Data were analyzed using the Statistical Package for the Social Sciences, Version 15.0 (SPSS Inc; Chicago, Illinois). Continuous variables are presented as mean ± SE. The χ^2 test was used to compare categorical variables between patients and controls.

The independent *t* test was applied to test differences in BDNF expression and other continuous variables between patients and controls. Parametric and nonparametric correlation analyses were used, as appropriate, to test the association between BDNF expression and psychosocial stress, inflammatory markers, and cortisol levels separately in patients and controls. A linear regression model was then used to test for predictors of BDNF expression and of hippocampal volume in patients.

RESULTS

Sociodemographic variables, psychosocial stress measures, and biological variables in first-episode psychosis patients and healthy controls are shown in Table 1. Patients with first-episode psychosis showed reduced BDNF gene expression (effect size, $d = 1.3$) and increased IL-6 ($d = 1.1$) and TNF-α ($d = 1.7$) gene expression compared with controls. Consistent with the data previously described in a sample comprising some of the same subjects,[22] measures of psychosocial stress were significantly higher in patients than in controls (Table 1), and cortisol levels were similar in patients and controls (Table 1).

Correlation Analyses With BDNF Gene Expression

We ran exploratory correlation analyses between BDNF gene expression levels and both the immune markers and the psychosocial stress measures in both patients and controls.

In patients, childhood trauma and number of recent stressful life events were negatively correlated with BDNF mRNA levels (respectively, Spearman $\rho = -0.42$, $P = .006$, and Spearman $\rho = -0.32$, $P = .03$). Interleukin-6 gene expression was also significantly negatively correlated with BDNF gene expression (Pearson $r = -0.33$, $P = .02$). Because of the presence of 1 outlier for IL-6 mRNA levels, we ran the analysis again without this subject, and the correlation remained significant ($r = -0.29$, $P = .05$). There was no significant correlation between BDNF mRNA levels and duration of antipsychotic treatment (see online supplementary eTable 1).

In the control group, there was a trend for a negative correlation between number of recent stressful events and BDNF gene expression (Spearman $\rho = -0.35$, $P = .06$). None of the other variables were correlated with BDNF gene expression in the control group (see online supplementary eTable 1).

Multiple Linear Regression Analyses for BDNF Gene Expression in First-Episode Psychosis

To establish possible predictors of BDNF gene expression in patients with first-episode psychosis, we ran a linear regression analysis including all the variables identified statistically to be correlated with BDNF mRNA levels. The first model included only the psychosocial stress variables (the number of childhood traumas and the number of recent stressful events) and accounted for 21% of the variance in BDNF gene expression (adjusted R^2, $P = .006$). Adding IL-6 gene expression to the model did not increase the variance explained, suggesting that the effects of psychosocial stressors on BDNF may be mediated by the increased IL-6; in fact, a second model including number of childhood traumas, number of recent stressful events, and IL-6 gene expression accounted for 23% of the variance in BDNF gene expression (adjusted R^2, $P = .009$) (Table 2).

Correlation Analyses With Left Hippocampal Volume in First-Episode Psychosis

In our previous article,[24] we reported that only the left hippocampal volume is correlated with cortisol levels during

Table 2. Multiple Linear Regression for Brain-Derived Neurotrophic Factor Expression in First-Episode Psychosis Patients

Linear Regression Model	Variables Included in Model	*R*	Adjusted R^2	*P* Value[a]
First	No. of childhood traumas No. of recent stressful events	0.51	0.21	**.006**
Second	No. of childhood traumas No. of recent stressful events Interleukin-6 expression	0.54	0.23	**.009**

[a]Boldface type indicates significance.

the day. This finding is consistent with studies showing that the left hippocampal volume is exquisitely sensitive to depression and psychosocial stressors[25,26] and that mainly the left-side hippocampal volume is smaller in first-episode psychosis.[41,42] Therefore, for this article, we conducted exploratory correlation analyses between left hippocampal volume and both the immune markers and the psychosocial stress measures. The BDNF and IL-6 mRNA levels and the diurnal cortisol levels all correlated with the left hippocampal volume (respectively, $r = 0.53$, $P = .01$; $r = -0.45$, $P = .04$; and $r = -0.68$, $P = .001$) (Figure 1). None of the other variables were correlated with left hippocampal volume (see online supplementary eTable 2).

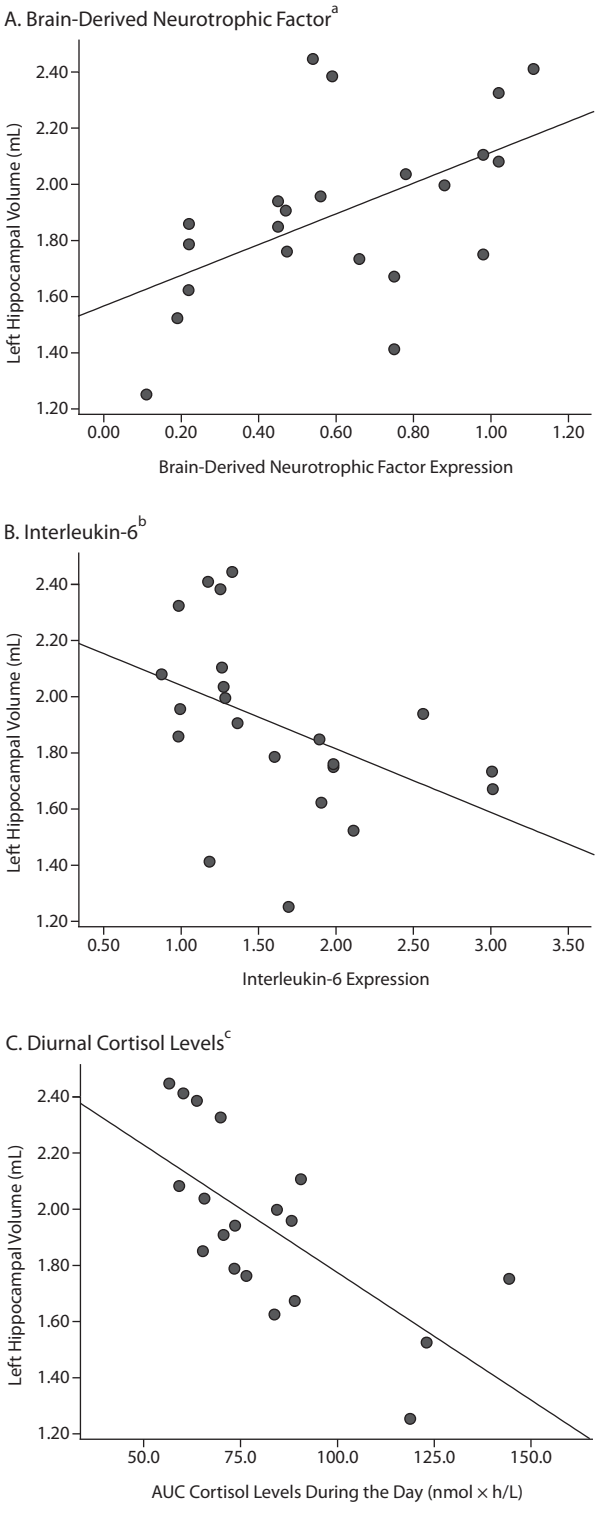
Multiple Linear Regression Analyses for Left Hippocampal Volume in First-Episode Psychosis

To establish possible predictors of (left) hippocampal volume in patients with first-episode psychosis, we ran a linear regression analysis including all the variables identified statistically to be correlated with this measure. We found that lower BDNF gene expression, higher IL-6 gene expression, and higher cortisol levels were *independently* associated with a smaller left hippocampal volume and that including 1, 2, or 3 variables in the models progressively increased the variance explained. The first model of the linear regression included only BDNF gene expression; this model accounted for 36% of the variance in left hippocampal volume (adjusted R^2, $P = .004$) (see Figure 1). The second model included BDNF and IL-6 gene expression; this model accounted for 47% of the variance in left hippocampal volume (adjusted R^2, $P = .002$). The third model included BDNF, IL-6 gene expression, and diurnal cortisol levels; this model accounted for 71% of the variance in left hippocampal volume (adjusted R^2, $P < .001$) (Table 3).

DISCUSSION

Our findings suggest that psychosocial stressors, both during childhood and closer to psychosis onset, play a role in reducing BDNF mRNA levels in leukocytes of patients with first-episode psychosis, possibly through a stress-induced increase in IL-6 expression. In turn, the reduced BDNF expression, together with the increased inflammation and the

Figure 1. Correlation of Left Hippocampal Volume With (A) Brain-Derived Neurotrophic Factor Gene Expression, (B) Interleukin-6 Gene Expression, and (C) Diurnal Cortisol Levels in First-Episode Psychosis Patients



[a]R^2 linear = 0.283.
[b]R^2 linear = 0.201.
[c]R^2 linear = 0.461.
Abbreviation: AUC = area under the curve.

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Table 3. Multiple Linear Regression for Left Hippocampal Volume in First-Episode Psychosis Patients

Linear Regression Model	Variables Included in Model	*R*	Adjusted R^2	*P* Value[a]
First	Brain-derived neurotrophic factor expression	0.63	0.36	**.004**
Second	Brain-derived neurotrophic factor expression Interleukin-6 expression	0.73	0.47	**.002**
Third	Brain-derived neurotrophic factor expression Interleukin-6 expression Diurnal cortisol levels	0.87	0.71	**<.001**

[a]Boldface type indicates significance.

increased cortisol levels, seems to contribute to the smaller left hippocampal volume in these patients.

Studies in animals have reported that early adverse experiences and chronic stress in adults lead to a persistent reduction of BDNF levels in various areas of the brain.[43–45] In contrast, an enriched early social environment has been shown to increase BDNF levels, especially in the hippocampus.[46] Interestingly, the very few data available in clinical populations seem to support the notion that psychosocial stress has a negative effect on BDNF levels. Indeed, 3 studies have found that the number of stressful events and the severity of childhood neglect are negatively correlated with peripheral BDNF levels in patients with affective disorders.[47–49] Our study is the first to report a negative effect of stressful events, both in childhood and closer to psychosis onset, on BDNF expression in patients with first-episode psychosis. Of note, these effects of psychosocial stressors on BDNF mRNA levels appear stronger in patients than in controls. Although findings in animal studies show that stress in itself is enough to affect BDNF levels,[43–45] the 3 aforementioned clinical studies[47–49] examining the effects of psychosocial stressors on BDNF are in samples of patients with depression or bipolar disorder. Therefore, it is possible that specific molecular pathways (including BDNF) are more vulnerable to being modified in response to stressful experiences in patients with psychosis (or, indeed, in patients with affective disorders) rather than in healthy subjects. However, patients in our sample also show higher rates of psychosocial stressors. Therefore, the larger number of events is another potential, not mutually exclusive, explanation for the stronger associations between stress and BDNF in patients than in controls.

Interestingly, this is also the first clinical study suggesting that the effects of psychosocial stressors on BDNF expression are mediated by increased levels of inflammation, as shown by the fact that the independent effect of IL-6 on BDNF levels disappears once the effects of psychosocial stressors are taken into account. Previous preclinical studies have proposed a number of mechanisms underlying the stress-related down-regulation of BDNF expression, including increased release of proinflammatory cytokines.[50] Indeed, administration of IL-1β has been shown to down-regulate BDNF expression in the rat hippocampus.[51,52] The mechanisms through which cytokines might influence BDNF expression are still unclear. Preclinical studies have suggested that proinflammatory cytokines could directly reduce BDNF gene transcription via pathways such as cyclic adenosine monophosphate–response element-binding protein phosphorylation or nuclear factor–κB activation.[53–55]

We also found a correlation between lower BDNF expression and smaller left hippocampal volume in first-episode psychosis. Our findings are consistent with previous studies[56,57] investigating the effect of BDNF Val66Met polymorphism on hippocampal volume. Specifically, these studies found a smaller hippocampal volume in healthy subjects and in patients with first-episode schizophrenia carrying the Met-BDNF allele, which has also been associated with lower depolarization-induced production of BDNF.[56,57] However, our data indicate that lower BDNF levels by themselves account for 36% of the variance of hippocampal volume. Adding IL-6 to the model increases the accounted for variance to 47%, while the best model predicting hippocampal volume in our patients includes BDNF, IL-6, and cortisol levels, accounting for more than 70% of the variance. This suggests that these 3 biological pathways *independently* contribute to the regulation of hippocampal volume. Interestingly, BDNF and proinflammatory cytokines can act on common intracellular pathways relevant to neuroplasticity, like the mitogen-activated protein (MAP) kinase cascade and the phosphotidylinositol-3 kinase/Akt signaling, and downstream regulators of apoptosis such as Bad (a major proapoptotic protein) and Bcl-2 (a major antiapoptotic protein).[58] Brain-derived neurotrophic factor and proinflammatory cytokines are also well known to exert an opposite effect on the regulation of the nuclear translocation of nuclear factor–κB, an important transcription factor decreasing cell survival.[59,60] Glucocorticoids influence neuroplasticity through their interaction with excitatory amino acid neurotransmitters and *N*-methyl-D-aspartate receptors.[61] Therefore, our findings suggest a synergic effect of BDNF, IL-6, and cortisol in determining smaller hippocampal volume, possibly through the activation of common molecular pathways.

From a clinical point of view, our findings further support the role of stress in the onset and outcome of psychosis, as already suggested by previous studies. Garner et al[62] reported a larger pituitary volume in people at high risk of developing psychosis who went on to develop psychosis within 1 year, suggesting that hypothalamic-pituitary-adrenal (HPA)–axis hyperactivity is already present before the onset of psychosis and predicts those who make the transition to psychotic episode. Moreover, a more recent study in drug-naive first-episode psychosis patients found that a larger pituitary volume at onset is associated with less improvement in psychotic symptoms after 12 weeks of antipsychotic treatment, further supporting the role of HPA-axis hyperactivity in the clinical outcome of these patients.[63] Furthermore, studies using the glucocorticoid antagonist mifepristone to target

HPA-axis activity have reported clinical improvement in patients with major psychotic depression.[64–66] More recently, drugs targeting the immune system, such as a COX-2 inhibitor (celecoxib) or a COX-1/COX-2 inhibitor (aspirin), have also been reported to significantly improve psychotic symptoms in patients with schizophrenia spectrum disorders when used as adjunctive therapy to antipsychotics.[67–70] Future pharmacologic studies should also take into account the relevance of reduced BDNF in psychosis as a possible future target for drug development.

A few limitations need to be acknowledged. First and foremost, our correlation analyses cannot unequivocally imply causation. Therefore, the observed association between high levels of stress and reduced BDNF expression could also have alternative interpretations. For example, through reversed causality, reduced BDNF expression could lead to the increased number of stressors through an effect on cognitive performance and personality traits in these patients.[12,71,72] Similarly, the smaller hippocampal volume may determine the higher cortisol and cytokine levels by reducing negative feedback of the HPA axis and inducing glucocorticoid resistance, a condition associated with inflammation.[73] Moreover, our correlation analyses were exploratory and were not corrected for multiple comparisons. Indeed, the large variance of hippocampal volume explained by the 3 combined stress biomarkers in the linear regression model is surprising, and these findings would need to be confirmed by future studies. A second limitation of this study is represented by our proposition that MRI volumes may represent a measure of neuroplasticity and neuronal function. Indeed, differences in MRI brain volumes might reflect alterations not only in neuronal cells, but also in nonneuronal tissue compartments (eg, changes in tissue perfusion, fat content, and water content).[74] Finally, only a subsample of patients (and none of the controls) agreed to undergo the brain MRI scan.

In conclusion, our findings suggest that the down-regulation of BDNF expression in first-episode psychosis is partly induced by childhood trauma and adulthood stressful events through a biological pathway that may involve increased inflammation. In turn, stress-related biological pathways, together with decreased BDNF expression, may account for the smaller left hippocampal volume. We believe that these biological pathways should be considered in the development of future therapeutic strategies for this condition.

Drug names: celecoxib (Celebrex), mifepristone (Mifeprex), olanzapine (Zyprexa).

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Supplementary material: Supplementary text and eTables are available at PSYCHIATRIST.COM.

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Supplementary Material

Article Title: Stress and Inflammation Reduce Brain-Derived Neurotrophic Factor Expression in First-Episode Psychosis: A Pathway to Smaller Hippocampal Volume

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List of Supplementary Material for the article

1. **Method Text** — Additional text for Method section of article, provided under the 3 following headings: Childhood Trauma Assessment, Magnetic Resonance Imaging, and Gene Expression Analyses

2. **eTable 1** — Exploratory Correlation Analyses With BDNF Gene Expression in First-Episode Psychosis Patients and in Healthy Controls

3. **eTable 2** — Exploratory Correlation Analyses With Left Hippocampal Volume in First-Episode Psychosis

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This Supplementary Material has been provided by the authors as an enhancement to the published article. It has been approved by peer review; however, it has undergone neither editing nor formatting by in-house editorial staff. The material is presented in the manner supplied by the author.

Supplementary Material

Method

Childhood Trauma Assessment

The modified version of Childhood Experience of Care and Abuse (CECA) Questionnaire included information about loss of parents, separation from parents for more than 6 months, and physical and sexual abuse occurred before the age of 17 years. A composite variable was created using the four dichotomized variables (loss of parents, separation from parents for more than 6 months, severe physical abuse and presence of sexual abuse): the score of this variable ranged between 0 (absence of any childhood trauma) to 4 (presence of all four childhood traumas investigated).

Magnetic Resonance Imaging

The whole brain was scanned with an axial inversion recovery prepared SPGR volume. TR was 11.2 ms, TI was 300 ms, TE was 4.8 ms, and the flip angle was 18 degrees, slice thickness was 1.1 mm. The images were obtained with in plane resolution 1.1mmx1.1mm, in 280x280 mm field of view.

Gene Expression Analyses

The RNA quantity was assessed by evaluation of the A260/280 and A260/230 ratios using a Nanodrop spectrometer (NanoDrop Technologies, Wilmington, DE, USA), and the RNA quality was determined using an Agilent Bioanalyzer (Agilent Technologies Italia S.p.A. Cernusco sul Naviglio, MI, Italy). Two micrograms of total RNA were then used for cDNA synthesis and for subsequent gene expression analysis in Real Time PCR. Quantitative Real-Time PCR was performed using HOT FIREPol® EvaGreen® qPCR Mix (Solis BioDyne, Tartu, Estonia) according to the

SYBR Green method. For each target primer set, a validation experiment was performed to demonstrate that PCR efficiencies were within the range of 90-100% and approximately equal to the efficiencies of the reference genes (glyceraldehyde 3-phosphate dehydrogenase (GAPDH), beta-actin (ACTB) and beta-2-microglobulin (B2M)). Briefly, after an initial heating step at 95°C for 15 min to activate the polymerase, 45 PCR cycles were performed. Each cycle consisted of a denaturation step at 95°C for 30 s, an annealing step at 60°C for 30 s and an elongation step at 72°C for 30 s. Each sample was assayed in duplicate and each target gene (BDNF, IL-6 and TNF-alpha) was normalized to the gene expression of the three reference genes: GAPDH, ACTB and B2M. The primers used to analyze BDNF gene expression levels have been designed in the BDNF coding region, therefore able to detect all the BDNF splice variants (primer Fw: TGGCTGACACTTTCGAACAC: primer Rw: AGAAGAGGAGGCTCCAAAGG). Pfaffl Method was used to determine relative target gene expression. Data were normalized to the geometric mean of all three reference genes and expressed as Relative Expression Ratio (R).

Results

eTable 1. Exploratory Correlation Analyses With BDNF Gene Expression in First-Episode Psychosis Patients and in Healthy Controls

	BDNF Expression in Patients	BDNF Expression in Controls
No. childhood traumas	**rho=-0.42, p=0.006**	rho=-0.1, p=0.6
No. recent stressors	**rho=-0.32, p=0.03**	rho=-0.35, p=0.06
Perceived stress scale	r=-0.23, p=0.1	r=0.01, p=1.0
IL-6 expression	**r=-0.33, p=0.02**	r=0.19, p=0.4
TNF-alpha expression	r=-0.09, p=0.6	r=0.27, p=0.2
Diurnal cortisol levels	r=0.07, p=0.7	r=-0.03, p=0.9
Days of antipsychotic treatment	r=0.06, p=0.7	—

eTable 2. Exploratory Correlation Analyses With Left Hippocampal Volume in First-Episode Psychosis

	Left Hippocampal Volume
No. childhood traumas	rho=0.27, p=0.3
No. recent stressors	rho=0.10, p=0.7
Perceived stress scale	r=0.09, p=0.7
IL-6 expression	**r=-0.45, p=0.04**
TNF-alpha expression	r=-0.31, p=0.2
BDNF expression	**r=0.53, p=0.01**
Diurnal cortisol levels	**r=-0.68, p=0.001**