



## Serum neurotrophin-3 is increased during manic and depressive episodes in bipolar disorder

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### Abstract

Accumulating evidence suggest that neural changes and cognitive impairment may accompany the course of bipolar disorder. Such detrimental effects of cumulative mood episodes may be related to changes in neurotrophins that take place during mood episodes but not during euthymic phases. The present study investigated serum neurotrophin-3 (NT-3) levels in patients with bipolar disorder during manic, depressed, and euthymic states, using an enzyme-linked immunosorbent assay (sandwich-ELISA). Serum NT-3 levels were increased in manic ( $p < 0.001$ ) and depressed ( $p < 0.001$ ) BD patients, as compared with euthymic patients and normal controls. These findings suggest that the NT-3 signaling system may play a role in the pathophysiology of BD.

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Bipolar disorder (BD) is a complex illness where episodes of mania alternate with depression and asymptomatic phases (euthymia). BD takes place in about 1% of the world population and is associated with increased mortality and burden [2]. While the pathophysiology of BD remains unclear, postmortem studies showed abnormal density and size of neuronal and glial cells in distinct subregions of the prefrontal cortex in patients with BD [11,14]. Such morphological changes suggest impairment in cellular plasticity and resilience rather than a neurodegenerative pattern [13]. In this context, there is increasing evidence suggesting that neurotrophic signaling systems, which regulate cellular plasticity and survival, may be altered in patients with major mood disorders [7]. For instance, infusions of BDNF and NT-3 into the dentate gyrus produce antidepressant effect in rodents [17], and the BDNF gene has been put forward as a sus-

ceptibility gene for BD [18]. Using peripheral blood, we [3,9,15] and others [20] have demonstrated that serum BDNF and GDNF are altered during major mood episodes in BD. As there are no available reports of serum NT-3 assessment in BD subjects, the aim of the present study was to evaluate serum NT-3 levels in BD patients during mania, depression and euthymia, as compared to age- and gender-matched controls.

The present study was approved by the local ethics committee (Hospital de Clínicas de Porto Alegre, Brazil) and all subjects provided written informed consent before entering in the study. Thirty euthymic, 19 depressed and 31 manic patients were consecutively recruited from the Bipolar Disorders Program, Hospital de Clínicas de Porto Alegre, Brazil and from the Inpatient Psychiatric Unit, Hospital Universitário de Santa Maria, Brazil. Diagnose was carried out using the Structured Clinical Interview for DSM-IV—Axis I (SCID-I) [4], and manic and depressive symptoms were assessed using the Young Mania Rating Scale (YMRS) [21] and the Hamilton Depression Rating Scale (HDRS) [6], respectively. Patients were considered

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Table 1  
Clinical and demographic characteristics of bipolar patients and healthy controls

	Healthy controls (n = 80)	Bipolar patients			p-value
		Euthymic (n = 30)	Manic (n = 31)	Depressed (n = 19)	
Gender					
Female	56.2%	50.0%	45.2%	48.0%	0.555*
Male	43.8%	50.0%	54.8%	52.0%	
Mean age (Years, S.D.)	41.73 (10.63)	41.63 (9.51)	39.53 (12.70)	43.42 (8.00)	0.638**
Number of medications (Mean, S.D.)	–	2.93 (1.23)	3.41 (1.40)	2.66 (0.96)	0.106**
Number of hospitalizations (Mean, S.D.)	–	4.69 (5.42)	4.39 (5.52)	7.26 (7.57)	0.241**
Number of suicide attempts (Mean, S.D.)	–	1.95 (1.70)	1.60 (0.89)	3.86 (3.03)	0.033**
Age of first episode (Mean, S.D.)	–	24.68 (11.60)	25.61 (10.92)	21.47 (10.36)	0.428**
Length of illness (Years, S.D.)	–	16.25 (10.39)	14.93 (11.21)	22.74 (10.71)	0.055**
CGI (Mean, S.D.)	–	3.53 (1.65)	5.55 (0.64)	4.05 (1.03)	0.001**
GAF (Mean, S.D.)	–	60.76 (13.45)	35.00 (7.30)	55.94 (9.76)	0.001**
HAM-D (Mean, S.D.)	–	5.65 (3.37)	11.46 (9.40)	16.89 (7.51)	0.001**
YMRS (Mean, S.D.)	–	5.16 (5.26)	34.19 (8.24)	5.15 (4.68)	0.001**

CGI: clinical global impression; GAF: global assessment of functioning; HAM-D: Hamilton depression rating scale; YMRS: Young mania rating scale.

\* Chi-square test.

\*\* One-way ANOVA test.

euthymic if they did not meet criteria for current manic or major depressive episode according to SCID-I and scored <7 on both YMRS and HDRS scales. Manic and depressive patients fulfilled SCID-I criteria for current manic or major depressive episode, respectively. The control group consisted of 80 healthy volunteers matched by age and gender, who manifested interest in participating in the study. Control subjects were not on any psychiatric or neurological medication, had no history of psychiatric or neurological diseases, and had no history of psychiatric or neurological conditions in their first and second-degree relatives.

Five milliliters of blood were withdrawn from each subject by venipuncture into a free-anticoagulant vacuum tube. Blood was immediately centrifuged at 3000 × g for 5 min, and serum was kept frozen at –80 °C until assayed. NT-3 serum levels were assessed using sandwich-ELISA from commercial kits which were handled according to the manufacturer's instructions (Chemicon, USA). Briefly, microtiter plates (96-well flat-bottom) were coated for 24 h with the samples diluted 1:2 in sample diluent and standard curve ranged from 7.8 to 500 pg of NT-3. Then, plates were washed four times with sample diluents and monoclonal anti-NT-3 rabbit antibody diluted 1:1000 in sample diluent was incubated for 2 h at room temperature. After washing, a second incubation with antirabbit antibody peroxidase conjugated diluted 1:1000 for 1 h at room temperature was carried out. After addition of streptavidin-enzyme, substrate, and stop solution the amount of NT-3 was determined (absorbance was set at 450 nm). The standard curve demonstrates a direct relationship between optical density (OD) and NT-3 concentration. We accepted intra- and inter-assay variation of ±4.0% and ±9.4%, respectively, as indicated by the manufacturer. Total protein was measured by Lowry's method using bovine serum albumin as a standard. Statistical Product and Service Solutions, version 12.0 (SPSS) was used to perform the statistical analysis. The Kolmogorov–Smirnov test was used to confirm that the results were normally distributed. Depressed, manic, and euthymic patients were compared to the control

group using a one-way ANOVA followed by Tukey post-hoc test when applicable.

Demographic and clinical characteristics of BD patients and controls are displayed in Table 1. There was no difference in age or gender between groups. All BD patients were on medication, but the number of medications, number of hospitalizations, age at first episode, and length of illness did not differ between groups. Depressed patients presented a higher number of suicide attempts, and manic patients had worse Clinical Global Impression (CGI) and Global Assessment of Functioning (GAF). Manic and depressive patients had higher serum NT-3 levels ( $F_{3,169} = 14.097$ ;  $p < 0.001$ ; see Fig. 1) than euthymic patients and controls. These results remained unchanged when controlled for potential confounders such as number of medications, number of suicide attempts, length of illness, GAF and CGI.

We showed that serum NT-3 is increased in BD subjects during manic and depressive episodes. To our knowledge, this is the first study to assess serum NT-3 levels in BD patients. These findings suggest increased production and/or secretion of NT-3 during acute mood states in BD. Recent studies suggest that levels of neurotrophic factors oscillate in an orchestrated fashion during the course of acute mood episodes and euthymic phases. For instance, serum BDNF is reduced in bipolar

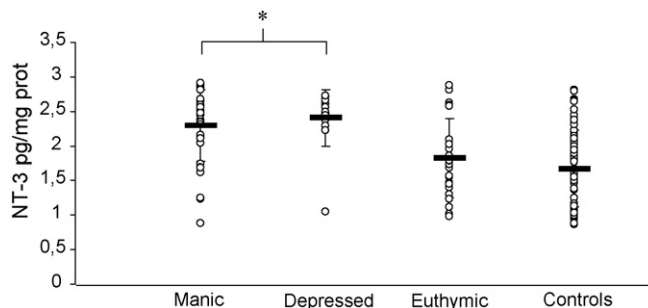


Fig. 1. Serum NT-3 levels in bipolar patients and healthy controls. Significantly different than euthymic and control groups ( $p < 0.01$ ; one-way ANOVA and Tukey post hoc).

depression and mania [3,9], while serum GDNF is increased during mania and bipolar depression [15]. It is noteworthy that neurotrophic factors seem to be altered only during the course of acute mood states, while stabilized (euthymic) patients present neurotrophic factors in the same levels as controls [3,15]. It has been reported that NT-3 is temporally and spatially expressed in the place of BDNF in some neuronal populations to compensate for the loss of BDNF [1]. These compensatory effects between NT-3 and BDNF were demonstrated by the fact that the critical role of these neurotrophins in the survival of hippocampal and cerebellar granule cells are revealed only when the signaling of both neurotrophins are blunted [10]. Further, NT-3 infusion increased BDNF mRNA expression in the cerebral cortex [16], and produces BDNF-like effects inducing cortical TrkB phosphorylation [5]. Therefore, we are tempted to speculate that this increment of serum NT-3 levels observed in the present study occurs in tandem with the lowering in BDNF serum levels described previously [3]. Such an increase in NT-3 could act as a compensatory physiological response to the strain imposed by mood episodes. This is in line with previous findings which showed that immobilization stress increased NT-3 mRNA expression in the dentate gyrus and locus coeruleus [19] and NT-3 infusion attenuated the correlates of disordered mood induced by the learned helplessness and forced swim test paradigms [17].

Some limitations of the present study should be addressed. First, we measured NT-3 levels in serum. Nevertheless, it has been demonstrated that NT-3 cross the brain–blood barrier, and there is a strong positive correlation ( $r=0.80$ ) between serum and brain NT-3 levels, suggesting that peripheral changes might correspond, at least in part, to central NT-3 changes [12]. Second, although it has been reported that antidepressants [19] but not antipsychotics [8] may affect NT-3 levels, little is known about the effects of other psychotropic agents on NT-3. Therefore, we cannot rule out the effects of medications in the present results. Further studies comparing NT-3 levels in medicated versus unmedicated BD patients would help clarify this issue. Another limitation is that this is a cross-sectional study. Longitudinal studies assessing BD subjects during, as well as outside acute episodes are warranted. In conclusion, we found that serum NT-3 levels are increased in BD patients during manic and depressive episodes, as compared with euthymic patients and healthy controls. The present findings suggest that NT-3 may be produced and/or secreted in response to the strain associated with acute mood episodes, and further support the current hypothesis that neurotrophins are involved in the pathophysiology of BD.

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