Neurotrophic factors in obsessive-compulsive disorder

Leonardo F. Fontenelle a,*, Izabela Guimarães Barbosa b, Juliano Victor Luna a, Natalia Pessoa Rocha b, Aline Silva Miranda b, Antonio Lucio Teixeira b

a Anxiety and Depression Research Program, Institute of Psychiatry, Federal University of Rio de Janeiro & D’Or Institute for Research and Education, Av. Venceslau Brás 71 Fundos, Botafogo, CEP 22290-140, Rio de Janeiro, Brazil
b Laboratory of Immunopharmacology, Institute of Biological Sciences, Federal University of Minas Gerais, Belo Horizonte, Brazil

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A B S T R A C T

In this cross-sectional study, we assessed the levels of neurotrophins (NF) of patients with obsessive-compulsive disorder (OCD) in different stages of treatment and their relationship with OCD clinical features. Forty patients with OCD and 40 healthy controls had Brain-Derived Neurotrophic Factor (BDNF), Nerve Growth Factor (NGF), and Glial Cell-Derived Neurotrophic Factor (GDNF) plasma levels measured by enzyme-linked immunosorbent assay (ELISA). Patients with OCD were further examined with the Obsessive-Compulsive Inventory-Revised, the Beck Depression Inventory, the Beck Anxiety Inventory, and the Sheehan Disability Scale (SDS). Patients with OCD exhibited significantly lower levels of BDNF and significantly increased levels of NGF as compared to healthy controls. In OCD, statistically significant negative correlations between BDNF levels and number of working days lost per week were found. Additional analyses revealed a statistically significant positive correlation between both NGF and GDNF and severity of washing symptoms. Plasma levels of NF were not affected by age, age at OCD onset, gender, major depressive disorder, the relative dose of serotonin-reuptake inhibitors being prescribed, or the use of antipsychotics. Our findings suggest that patients with OCD may exhibit a particular NF profile, with functional impairment correlating with BDNF levels and severity of washing symptoms correlating with NGF and GDNF levels.

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1. Introduction

The serotonergic hypothesis of obsessive-compulsive disorder (OCD), proposed more than 30 years ago (Yaryura-Tobias, 1977), was largely derived from the superiority of clomipramine over less serotonergic drugs in the treatment of OCD (Yaryura-Tobias, 1977; Piccinelli et al., 1995). Since then, a number of studies have supported a key role of the brain serotonin (5-HT) system in the pathophysiology of OCD. These findings include the exacerbation of obsessive-compulsive symptoms following the administration of m-chloro-phenyl-piperazine (an agonist to 5-HT2C, 5HT1A, and 5-HT1D receptors) (Zohar et al., 1987; Pigott et al., 1991; Hollander et al., 1992; Khanna et al., 2001) and sumatriptan (an agonist to 5-HT1D receptors) (Koran et al., 2001; Gross-Isseroff et al., 2004), the elevation of 5-hydroxyindolacetic acid levels in the cerebrospinal fluid of patients with OCD (Insel et al., 1985; Stengler-Wenzke et al., 2004), the differential densities of the 5-HT transporter in specific regions OCD patients’ brains (Pogarell et al., 2003; Hesse et al., 2005; Reimold et al., 2007; Zitterl et al., 2007), the increased radioactive Methyl-L-Tryptophan trapping in the right hippocampus, the left inferior temporal gyrus, and bilateral caudate nuclei in medication-free patients with OCD (Berney et al., 2011), and the association between the long allele of the 5HT transporter with specific OCD subgroups, such as childhood-onset OCD and Caucasian subjects, as shown in a meta-analysis of 18 studies involving 2283 patients (Bloch et al., 2008).

Although the serotonergic hypothesis of OCD has found support in the literature, additional studies suggest that other neurotransmitters, such as dopamine (DA), may also be involved in the pathophysiology of OCD. For instance, patients with OCD who respond partially to 5HT reuptake inhibitors (SRI) benefit from D2 blocking drugs (Bloch et al., 2006), dopaminergic drugs lead to OCD-like behaviors in animals (Elam and Szechtman, 2005) and humans (Crum and Anthony, 1993; O’Sullivan et al., 2010), and neuroimaging studies have revealed DA transporter binding abnormalities (van der Wee et al., 2004; Pogarell et al., 2005; Kim et al., 2007) and reduced D1 binding potential in drug-free OCD patients (Olver et al., 2009, 2010) as compared to matched healthy controls. One study showed blunted growth hormone response to acute apomorphine in OCD patients (Brambilla et al., 1997). Finally, a recent meta-analysis (Pooley et al., 2007) provided significant
Neurotrophic factors (NF) belong to families of structurally and functionally related molecules including the neurotrophin super-family [which subsumes the brain-derived neurotrophic factor (BDNF), the nerve growth factor (NGF), the neurotrophin-3 (NT-3) and the neurotrophin-4/5 (NT-4/5)] and the glial cell line-derived neurotrophic factor (GDNF) family, among others (Barde, 1990). They are a unique family of polypeptides that influence axon growth, dendrite pruning, and the expression of proteins crucial for normal neuronal function, such as neurotransmitters and ion channels (Huang and Reichardt, 2001). Of note, BDNF, NGF and GDNF and have been shown to interact reciprocally with different circuits and neurotransmitters, including 5HT (Martinowich and Lu, 2008), DA (Carnicella and Ron, 2009), and acetylcholine (ACh) (Cirulli and Alleva, 2009) systems, respectively. For instance, Lyons et al. (1999) followed heterozygous BDNF (+/-) mice and showed a range of impulsive behaviors (i.e. aggressiveness and hyperphagia) that correlated with decrements in forebrain 5-HT levels and fiber density in early adulthood and were ameliorated by SRI treatment. Clearly, it is plausible that different NF and neurotransmitter systems influence each other to regulate the development and plasticity of neural circuits involved in anxiety disorders, such as OCD.

To date, only few studies have assessed NF (i.e. BDNF) levels in patients with OCD (Yoshimura et al., 2006; Maina et al., 2010; Wang et al., 2011; Dos Santos et al., 2011). In a case series, Yoshimura et al. (2006) were unable to find variations in BDNF levels after drug-treatment and recovery from OCD symptoms. Maina et al. (2010) reported marginally decreased serum levels of BDNF in 24 drug-naïve OCD patients as compared with 24 controls, but were unable to find correlations between BDNF levels and symptoms severity, age at onset of OCD, length of illness, family history, and quality of life.

Similarly, Wang et al. (2011) compared BDNF plasma levels in 22 drug-naïve OCD patients without a lifetime history of depression, 52 drug-treated OCD patients and 63 healthy controls. They found that BDNF plasma levels in both OCD groups were lower than those in normal controls but did not differ between each other. In this study, although no significant relationship between BDNF plasma levels and illness severity, age, age at onset of symptoms, length of illness, or drug dosage was found, length of drug treatment was positively associated with BDNF plasma levels.

More recently, Dos Santos et al. (2011) found that serum BDNF levels of 25 drug-free (but not necessarily treatment naïve) OCD patients were lower than those exhibited by 25 controls. They also found that higher plasma BDNF levels were associated with the presence and severity of the sexual/religious OCD symptoms and, in a counterintuitive manner, with the co-occurrence of different stages of treatment and in age-, gender-, and education-paired healthy controls. More specifically, we predicted that patients with OCD would exhibit alterations in NF plasma levels and also explored the relationships between different OCD symptom dimensions and these levels.

2. Methods

2.1. Subjects

Forty consecutive patients with OCD who sought treatment at Anxiety and Depression Research Program of the Institute of Psychiatry of the Federal University of Rio de Janeiro were selected according to the following inclusion criteria: (1) having DSM-IV-OCD as their primary psychiatric diagnosis (i.e. the one displaying the earliest onset and greatest severity) as reported by their attending physician, (2) age between 18 and 65 years, and (3) having the ability to read and fill out forms. Subjects with neurodegenerative, infectious or autoimmune diseases, or who had used steroids, anti-inflammatory drugs or antibiotics in the 4 weeks before venipuncture were preliminarily excluded from this research protocol. Patients with a recent history of stressful life events were not excluded. For the sake of comparison, 40 healthy age and gender-matched subjects were recruited from community as controls. The local institutional review board approved the study, which is in accordance with the Helsinki Declaration of 1975. All volunteers provided their written consent after a complete explanation about the procedures involved in the research protocol. Patients and healthy controls were assessed with the Mini-International Neuropsychiatric Interview (M.I.N.I.-Plus) to confirm OCD and other psychiatric disorders (in controls) and to exclude a history of psychiatric disorders (in controls) (Amorim, 2000). Patients with OCD were also examined with the Obsessive-Compulsive Inventory-Revised (OCI-R) (Sohuz et al., 2011), the Beck Depression Inventory (BDI) and Beck Anxiety Inventory (Cunha, 2001), and the Sheehan's Disability Scale (SDS) (Sheehan et al., 1996). To rate the relative dose of SRI or other antidepressants being used by OCD patients, scores were attributed to therapeutically equivalent doses across different medications, i.e. 0 to no medication at all, 1–20 mg of fluoxetine, paroxetine or citalopram, 50 mg of sertraline, 100 mg of fluvoxamine, or 75 mg of clomipramine, 2–40 mg of fluoxetine, paroxetine or citalopram, 100 mg of sertraline, 200 mg of fluvoxamine, or 150 mg of clomipramine, and so on.

2.2. Procedure

Ten milliliters of blood were drawn between 8 and 10 a.m. from each subject by venipuncture into a sodium heparin tube, at the moment of the clinical assessment. The blood was immediately centrifuged at 3.000 g for 10 min, 4 °C, twice. The plasma was collected and stored at −70 °C until assayed. Plasma levels of BDNF, GDNF and NGF were measured by enzyme-linked immunosorbent assay (ELISA) according to the manufacturer procedure (R&D Systems, Minneapolis, MN, USA). All samples were assayed in duplicate. Concentration was expressed as pg/mL. Detection limits were defined as 5 pg/mL for BDNF and 10 pg/mL for NGF and GDNF.

2.3. Statistical analysis

Descriptive statistics were used to report socio-demographic and clinical characteristics of the sample. Data were presented as mean ± standard deviation (S.D.), median, interquartile range, or percentage, as indicated. All variables were tested for normal distribution by means of the Kolmogorov–Smirnov test and were non-normally distributed. Relationships between dichotomous variables were assessed with the χ² test or the Fisher’s exact test when appropriate.

For continuous variables, differences between patients and controls were compared with the Mann–Whitney’s test. Spearman’s rank-order correlation analysis was performed to examine the relationship among age, age at OCD onset, number of working days lost or unproductive days per week, severity of symptoms, and individual symptoms. All statistical tests were two-tailed and the level of significance level was set at p < 0.05. Statistical analyses were performed using SPSS software version 17.0 (SPSS Inc., Chicago, IL, USA).

3. Results

Demographic and clinical features of all subjects are shown in Table 1. There were no significant differences between OCD patients and control group regarding age, gender and years of study. At the time of the interview, the mean length of OCD was 20.27 years (S.D. ± 11.48). Twenty-nine patients (72.5%) presented one or more psychiatry comorbidities. The most frequent
comorbidity was major depressive disorder, which affected 21 patients (52.5%). Thirty-seven patients (94.9%) were under treatment with SRIs, 10 of whom (30.3%) were also in use of antipsychotics.

OCD patients presented lower BDNF plasma levels (median interquartile range) pg/ml: 520.85 [412.34–791.31] and 874.92 [508.44–1668.57], respectively, Z = −2.99; p = 0.003) (Fig. 1A) and higher NGF plasma levels than controls (median interquartile range) pg/ml: 119.46 [75.98–192.93] and 80.25 [39.59–173.65], respectively, Z = −1.96; p = 0.05) (Fig. 1B). There was no difference between GDNF plasma levels in OCD patients and controls (Z = −1.14; p = 0.25) (Fig. 1C).

In the OCD sample, plasma levels of BDNF, NGF, and GDNF did not differ according to age (p = 0.15; p = 0.33; p = 0.003, p = 0.98; and p = 0.19, p = 0.24, respectively), age at OCD onset (p = 0.13; p = 0.42; p = −0.19, p = 0.24; and p = −0.19, p = 0.25, respectively), gender (Z = −1.68; p = 0.11; Z = −0.63; p = 0.52; and Z = −0.28; p = 0.77, respectively), presence of major depression (Z = −0.34; p = 0.73; Z = −0.63; p = 0.52; and Z = −0.77; p = 0.44, respectively), relative dose of antidepressant being prescribed (p = −0.21; p = 0.23; p = −0.007; p = 0.96; and p = −0.08; p = 0.66, respectively), or the use of antipsychotics (Z = −1.30; p = 0.19; Z = −0.41; p = 0.97; and Z = −0.12; p = 0.90, respectively). When the analyses were restricted to healthy controls, females had higher plasma levels of BDNF than males (Z = −3.07; p = 0.002), but no difference between genders were found in terms of NGF (Z = −1.37; p = 0.17) and GDNF levels (Z = −0.28; p = 0.77).

Among OCD patients, BDNF plasma levels correlated significantly with working days lost per week (p = −0.33, p = 0.04) and, on a trend level, with total SDS (p = −0.29, p = 0.07). Plasma levels of NGF and GDNF correlated significantly with severity of washing symptoms (p = 0.33, p = 0.04; and p = 0.33, p = 0.04, respectively). Also, while NGF correlated, on a trend level, with unproductive days per week (p = −0.29, p = 0.07), GDNF plasma levels correlated, on a trend level, with severity of ordering symptoms (p = 0.30, p = 0.07).

There were no additional relationships between BDNF, GDNF and NGF plasma levels and other clinical and demographic parameters evaluated, both in OCD patients and in controls (Table 2).

4. Discussion

In this study, we found that patients with OCD exhibited significantly lower levels of BDNF and significant increased levels of NGF as compared with healthy controls. Further, when analyses were restricted to patients with OCD, a number of additional findings emerged. Firstly, we found a statistically significant negative correlation between BDNF levels and number of working days lost per week and a trend towards a negative correlation

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Table 1
Comparisons between sociodemographic features displayed by patients with OCD and controls.

<table>
<thead>
<tr>
<th></th>
<th>OCD patients (n=40)</th>
<th>Healthy controls (n=40)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender (Female/Total)</td>
<td>19/40</td>
<td>19/40</td>
<td>1.00a</td>
</tr>
<tr>
<td>Age in years mean (± S.D.)</td>
<td>38.25 (± 13.32)</td>
<td>38.10 (± 10.05)</td>
<td>0.66b</td>
</tr>
<tr>
<td>Educational levels in years mean (± S.D.)</td>
<td>15.13 (± 2.68)</td>
<td>13.85 (± 4.28)</td>
<td>0.32b</td>
</tr>
<tr>
<td>OCI-R (± S.D.)</td>
<td>26.36 (± 14.70)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BDI (± S.D.)</td>
<td>18.50 (± 10.49)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>BAI (± S.D.)</td>
<td>16.76 (± 10.95)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>SDS (± S.D.)</td>
<td>12.87 (± 10.56)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Number of working days lost per week, mean (± S.D.)</td>
<td>1.26 (± 2.47)</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Number of unproductive days per week, mean (± S.D.)</td>
<td>1.12 (± 2.09)</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

Abbreviations: OCD: obsessive-compulsive disorder; S.D.: standard deviation; OCI-R: Obsessive-Compulsive Inventory-Revised; Beck Depression Inventory; BAI: Beck Anxiety Inventory; SDS: Sheehan Disability Scale.

a Chi-square’s test.
b Mann-Whitney’s test.
between BDNF levels and scores on the SDS. Secondly, we found a statistically significant positive correlation between both NGF and GDNF levels and the severity of washing symptoms. Finally, there was a hint for a negative correlation between NGF and number of unproductive days and for a positive correlation between GDNF and the severity of washing symptoms. Finally, there was a hint for a negative correlation between NGF and number of unproductive days and for a positive correlation between GDNF and the severity of washing symptoms. Furthermore, a positive correlation between both NGF and GDNF was found in patients with OCD with different conditions, including OCD (Bus et al., 2011).

Increased levels of NGF among patients with OCD, which correlated with severity of washing symptoms significantly and with the number of unproductive days on a trend level, can be interpreted in at least two different but not mutually exclusive ways. Firstly, increased NGF levels could represent a coping response involved in preparing the organism to face a highly stressful situation (Aloe et al., 1994). For instance, in a study with first time parachute jumpers, while cortisol and ACTH rose only after landing, increased levels of NGF were already noted in the evening before jumping. This interpretation is consistent with studies showing that, among different OCD cognitions, overestimation of threat was most strongly correlated to contamination/washing symptoms (No authors listed, 2001, 2005). Secondly, our findings suggest that OCD washing may be associated with some sort of cholinergic dysfunction, since the highest brain levels of NGF are found in targets for basal forebrain cholinergic neurons (e.g. the septo-hippocampal system) (Korsching et al., 1985; Large et al., 1986). These findings also dovetail with studies suggesting the occurrence of a cholinergic supersensitivity in OCD (Lucey et al., 1993) and the anti-OCD superiority of anticholinergic vs. non-cholinergic SRIs (Piccinelli et al., 1995).

We also found that GDNF correlated positively with severity of washing. Notably, it has been speculated that GDNF, which is found in high levels in Nucleus Accumbens (NAcc) and is retrogradely transported to the substantia nigra pars compacta and the ventral tegmental area (Barroso-Chinea et al., 2005), plays a unique role in regulating motivation and drive (Carnicella and Ron, 2009). Coherently, in a functional magnetic resonance imaging using a monetary incentive delay task, OCD washers exhibited significantly reduced NAcc activity during reward anticipation as compared to OCD checkers (Denys et al., 2010). Considering the close relationship between GDNF levels and dopaminergic activity (Carnicella and Ron, 2009), the fact that GDNF correlated with severity of ordering and symmetry symptoms is consistent with previous findings suggesting greater dopaminergic involvement in these patients, such as greater resistance to selective SRIs (Stein et al., 2007), increased response to monoamineoxidase inhibitors (Jenike et al., 1997) and higher rates of comorbid Tourette syndrome (Baer, 1994).

Admittedly, our study has a number of limitations. Most importantly, to differentiate our study from the previous assessment of BDNF levels in OCD (Maina et al., 2010), we have performed an exploratory analysis of a treatment-seeking sample that included patients with OCD who were under medication, but did not collect data on duration of the treatment. Indeed, that information would be relevant, particularly considering that length of drug treatment was positively associated with BDNF plasma levels in a previous OCD study (Maina et al., 2010). In spite of this, one must consider that an attempt to control the effects of SRI on NF levels was performed by taking into account equivalent doses of SRI being administered, but no association between SRI and NF emerged. There is also no reason to believe that SRI treatment could explain our findings, since the effects of antidepressants in the opposite way are largely documented (Brunoni et al., 2008). Finally, neither depression, nor use of antipsychotics, could explain different NF levels among patients with OCD.

### Table 2

<table>
<thead>
<tr>
<th></th>
<th>BDNF</th>
<th>NGF</th>
<th>GDNF</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.03</td>
<td>0.24</td>
<td>0.19</td>
</tr>
<tr>
<td>Age of onset</td>
<td>0.34</td>
<td>0.37</td>
<td>0.25</td>
</tr>
<tr>
<td>Length of disease</td>
<td>0.26</td>
<td>0.25</td>
<td>0.64</td>
</tr>
<tr>
<td>Sheehan’s Disability Scale</td>
<td>0.24</td>
<td>0.32</td>
<td>0.42</td>
</tr>
<tr>
<td>Unproductive days per week</td>
<td>0.07</td>
<td>0.14</td>
<td>0.07</td>
</tr>
<tr>
<td>Work days lost per week</td>
<td>0.04</td>
<td>0.07</td>
<td>0.01</td>
</tr>
<tr>
<td>Beck Depression Inventory</td>
<td>0.02</td>
<td>0.15</td>
<td>0.09</td>
</tr>
<tr>
<td>Beck Anxiety Inventory</td>
<td>0.03</td>
<td>0.20</td>
<td>0.09</td>
</tr>
<tr>
<td>Checking</td>
<td>0.02</td>
<td>0.08</td>
<td>0.08</td>
</tr>
<tr>
<td>Hoarding</td>
<td>0.04</td>
<td>0.17</td>
<td>0.03</td>
</tr>
<tr>
<td>Neutralizing</td>
<td>0.04</td>
<td>0.50</td>
<td>0.02</td>
</tr>
<tr>
<td>Obsession</td>
<td>0.06</td>
<td>0.01</td>
<td>0.02</td>
</tr>
<tr>
<td>Ordering</td>
<td>0.05</td>
<td>0.32</td>
<td>0.01</td>
</tr>
<tr>
<td>Washing</td>
<td>0.04</td>
<td>0.33</td>
<td>0.01</td>
</tr>
</tbody>
</table>

Rho—Spearman’s rank correlation coefficient.
In sum, our findings suggest that patients with OCD may exhibit a particular NF profile, with functional impairment correlating with BDNF levels and severity of washing symptoms correlating with NGF and GDNF levels.

Statement of interest

None.

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