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Research Letter

Brain-derived neurotrophic factor in romantic attachment

Introduction

Social attachment is fundamental for its relevant impact upon survival and reproduction in several animals and particularly so in humans.

Recently, much research efforts have been directed towards the understanding of the neurobiological basis of social attachment. Besides oxytocin, vasopressin and specific brain areas, it has also been suggested that neurotrophins (NTs) might be involved. NTs are a family of structurally similar proteins including nerve growth factor (NGF) and brain-derived neurotrophic factor (BDNF) amongst others, which play an important role in the survival, differentiation and functioning of neurons (Patapoutian & Reichardt, 2001). Preclinical studies suggest that BDNF might be involved in hippocampal degeneration (Duman *et al*. 1997) following different stressors, such as maternal deprivation (Meaney, 2001). The BDNF changes would render adult animals more vulnerable to exaggerated stress responses and anxious behaviours (Ladd *et al*. 2004). These findings suggest that BDNF, and perhaps all NTs, might act as mediators to translate the effects of environmental stimuli on the development of the 'social brain'.

A type of social attachment that seems to be peculiar to humans is the so-called 'romantic attachment', which is the establishment of a relationship between two sexual partners (Hazan & Shaver, 1987). Given the lack of information on this topic, we explored the possible relationship between BDNF plasma levels and romantic attachment, as assessed by the Experiences in Close Relationships (ECR) questionnaire (Brennan *et al*. 1998), a self-report instrument for measuring romantic attachment in adults.

Subjects and methods

Subjects

Twenty-four healthy subjects (12 men, 12 women, mean age 30.8 ± 4.9 years) agreed to participate in this study. They were residents, post-doctoral fellows or clinicians at the Speciality School of Psychiatry, University of Pisa. No subject had a family or personal history of any major psychiatric disorder, or had ever regularly taken psychotropic drugs, as assessed by a detailed psychiatric interview. All were free of physical illness, were neither heavy cigarette smokers, nor taking any regular medication. The women had normal menstrual cycles and were not taking contraceptive pills; their blood was drawn in the early follicular phase. The men had no history of genital disease or hypogonadism. Prior to enrolment, subjects gave their written informed consent to participate in the study. The study was approved by the Ethics Committee of the University of Pisa.

Instruments

Romantic attachment was assessed using the Italian version of the ECR (Picardi *et al*. 2000). It consists of 36 items, scored on a seven-point Likert scale, with 1 indicating 'completely false' and 7 indicating 'completely true'. In addition, it provides two scale scores measuring anxiety and avoidance, the two main dimensions underlying adult attachment styles. According to the scores on these scales, subjects can be classified in terms of four mutually exclusive categories of attachment. Participants scoring above normal on the anxiety scale were classified as preoccupied, those scoring above normal on the avoidance scale were classified as dismissing, and those scoring above normal on both scales were classified as fearful/avoidant, while all the remaining participants were classified as secure.

Methods

During January and February 2007, between 0800 and 0900 hours, blood (20 cc) was drawn from fasting subjects who were relaxing in the same room at a constant temperature. Blood was collected into EDTA-coated tubes that were kept on ice, centrifuged at 2000 *g* for 10 min at 4 °C and refrigerated at −20 °C. To measure the amount of total BDNF, acidification and subsequent neutralization of the samples were performed before proceeding with the enzyme-linked immunosorbent assay (ELISA) protocol, according to manufacturer's instruction (Promega, Switzerland). Ninety-six-well plates were coated with anti-BDNF monoclonal antibody and incubated at 4 °C for 18 h. The plates were then incubated in a blocking buffer for 1 h at room temperature, and the samples were added.

Table 1. *Characteristics of the subjects, distribution of the attachment styles, BDNF plasma levels, ECR total score and comparisons between genders*

	All subjects ($n=24$)		Women ($n=12$)		Men ($n=12$)		Test	
	n	(%)	*n*	(%)	*n*	(%)	χ^2	*p*
Marital status								
Single	16	(66.7)	7	(58.3)	9	(75.0)	0.917	0.63
Married	6	(25.0)	4	(33.0)	2	(16.7)		
Separated/divorced	2	(8.3)	1	(8.3)	1	(8.3)		
Attachment Styles (ECR)								
Secure	11	(45.8)	5	(41.7)	6	(50.0)	2.924	0.40
Preoccupied	8	(33.3)	5	(41.7)	3	(25.0)		
Fearful/avoidant	3	(12.5)	2	(16.7)	1	(8.3)		
Dismissing	2	(8.3)	0	(0)	2	(16.7)		
	Mean	S.D.	Mean	S.D.	Mean	S.D.	*t*	
Age (years)	30.8	(4.9)	29.3	(3.1)	32.2	(6.1)	1.483	0.15
ECR anxiety scale	3.7	(0.9)	4.3	(1.1)	3.1	(0.9)	−2.805	0.01
ECR avoidance scale	2.1	(0.9)	2.0	(1.1)	2.2	(0.7)	0.486	0.63
Plasma BDNF (ng/ml)	6.4	(3.1)	7.4	(2.5)	5.3	(3.4)	−1.798	0.09

BDNF, Brain-derived neurotrophic factor; ECR, Experiences in Close Relationships questionnaire.

The samples and BDNF standards were maintained at room temperature under shaking for 2 h, followed by washing with the appropriate buffer. The plates were successively incubated with anti-human BDNF polyclonal antibody at room temperature for 2 h, washed, and incubated with anti-IgG antibody conjugated to horseradish peroxidase for 1 h at room temperature. The plates were incubated in peroxidase substrate and tetramethylbenzidine solution to produce a colour reaction. The reaction was stopped with 1 M HCl. The absorbance at 450 nm was measured with a microplate reader (Model 550, Bio-Rad Laboratories, USA) to determine BDNF values that are expressed as pg/ml.

Statistical analyses

As BDNF data were not normally distributed, as verified by the Kolmogorov–Smirnov test, they were logarithmically transformed to accommodate a normal distribution. BDNF levels were compared between genders using the *t* test (two-tailed, unpaired) and between subjects with different attachment styles and demographic characteristics with ANOVA. Correlations between the levels of BDNF and age, duration of the romantic relationship, and anxiety or avoidance scales of the ECR were examined using Pearson's correlation coefficient. Correlations between the levels of BDNF and the score of each single item of the ECR were performed by means of Spearman's correlation coefficient. All data are presented as mean ± S.D. The significance level was set at $p<0.05$. All analyses were performed using SPSS version 12.1 (SPSS Inc., USA).

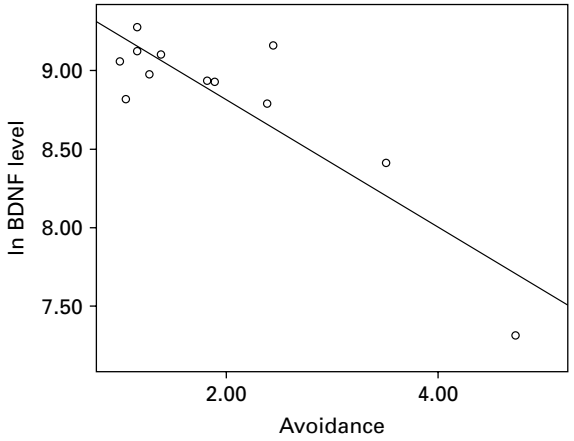

Fig. 1. The relationship between brain-derived neurotrophic factor (BDNF) plasma levels and the Experiences in Close Relationships (ECR) avoidance scale total in women (ln BDNF level = 9.63 − 0.40 × avoidance; $R^2=0.77$).

Results

Table 1 shows the demographic characteristics of the subjects, the scores of the ECR scales, the distribution

of different attachment styles, as well as the BDNF plasma levels (mean ± S.D.) in the whole sample and the comparisons between genders.

Women and men were not different, except that women showed a significantly higher score on the ECR anxiety scale than men ($t = -2.805$, $p = 0.010$).

A significant and negative correlation was observed between the ECR avoidance scale and BDNF plasma levels, but only in women ($r = -0.87$, $p = 0.001$) (Fig. 1). On the contrary, the other continuous parameters (age, length of the relationship, ECR anxiety scale) were unrelated to this biological parameter; moreover, no significant relationships were found between categorical variables, such as marital status or attachment styles, and BDNF. Significant and negative correlations were observed in women between BDNF levels and single ECR items, in particular no. 1 ('I prefer not to show a partner how I feel deep down') ($r_s = -0.66$, $p = 0.021$) and no. 13 ('I am nervous when partners get too close to me') ($r_s = -0.614$, $p = 0.034$). Positive correlations were measured with no. 29 ('I feel comfortable depending on romantic partners') ($r_s = 0.565$, $p = 0.05$), no. 31 ('I don't mind asking romantic partners for comfort, advice or help') ($r_s = 0.755$, $p = 0.055$) and no. 33 ('It helps to turn to my romantic partner in times of need') ($r_s = 0.564$, $p = 0.056$).

Discussion

The results of the present study showed that BDNF plasma levels and romantic attachment, assessed by the ECR, are related, but differently in the two sexes. In fact, women showed a significant and negative correlation between BDNF levels and the ECR avoidance scale, i.e. the higher the NT concentration, the lower the avoidance score. This suggests that BDNF may play a role in promoting social relationships through a specific decrease of avoidance and fear of the stranger and unfamiliar individuals: this is consistent with the recently proposed involvement of BDNF in reducing social stress responses, as shown by the association between BDNF Val^{66}Met polymorphism and vulnerability to social stress (Shalev *et al*. 2009). It has been hypothesized that the BDNF gene, and perhaps NGF, may regulate the HPA axis response to psychological stress, while protecting neurons against stress-induced damage (Bergström *et al*. 2008). Interestingly, NGF levels have been shown to be significantly higher in those subjects who had recently fallen in love, compared to subjects who were single or engaged in a long-lasting relationship (Emanuele *et al*. 2006). However, it is unclear why BDNF plasma levels and the avoidance of romantic attachment are related only to women, as the men of our sample lacked this correlation. It can be hypothesized that the role of BDNF in stress responses might be gender-related, and based on hormonal and genotype interactions. Recently, oestrogens have been implicated in inducing BDNF synthesis in several brain regions (Sasahara *et al*. 2007). It seems that BDNF should be 'more active' in women than in men, as they are basically more anxious (Kessler *et al*. 2001; Afifi, 2007); not surprisingly, the ECR scores on the anxiety scale of the women in our sample were higher than those of the men.

We observed several correlations with single items, in particular, negative with no. 1 ('I prefer not to show a partner how I feel deep down') and no. 13 ('I am nervous when partners get too close to me'), and positive with no. 29 ('I feel comfortable depending on romantic partners'), no. 31 ('I don't mind asking romantic partners for comfort, advice or help'), and no. 33 ('It helps to turn to my romantic partner in times of need'). Such correlations in the opposite direction support the role of BDNF in promoting subjective feelings of well-being when the partner is present and is considered reliable.

Some bias of this study should be acknowledged. First, the small sample size and the similar characteristics of the subjects, so that the findings may not be generalized. In any case, we controlled some possible confounding factors, such as age, time of the day and menstrual cycle in women (Piccinni *et al*. 2008). Another limitation concerns the reliability of plasma BDNF levels as a peripheral mirror of central concentrations. However, this parameter is thought to represent a reliable and sensitive marker of its variations occurring in the brain (Lommatzsch *et al*. 2005).

In conclusion, in our opinion, the findings reported herein are intriguing, as they represent the first observations of links between BDNF and romantic attachment features in a sample of healthy subjects. Further studies should explore whether BDNF, and other NTs, may be related to other human social relationships.

Declaration of Interest

None.

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Research Letter

High-density negative ion treatment increases positive affective memory

Acute administration of antidepressant drugs which potentiate the activity of serotonin (5-HT) and/or noradrenaline (NA) produces positive biases in the processing of emotional information in healthy volunteers (Harmer *et al*. 2003*a*, *b*; Browning *et al*. 2007). Because this action could be relevant to the way in which antidepressant drugs produce their therapeutic effects it is important to find out whether other antidepressant treatment modalities also positively bias emotional processing. Preliminary evidence suggests that high-density negative ion (HDNI) treatment produces antidepressant effects in well-controlled small-scale studies of patients with winter depression in which it is as efficacious as bright-light treatment (Terman *et al*. 1998; also see Terman & Terman, 2006). In addition, high-density ions appear effective in the treatment of chronic depression (Goel *et al*. 2005) and also in improving mood in mildly depressed students even with acute exposure (Goel & Etwaroo, 2006). We therefore assessed the effect of a single session of HDNI treatment on models of emotional processing in healthy volunteers.

We studied 30 healthy participants (17 females, 13 males, aged between 18 and 28 years) who were screened to be free of current or previous Axis I psychiatric disorder on the Structured Clinical Interview for DSM-IV (SCID-IV). None of the participants were