Raised plasma nerve growth factor levels associated with early-stage romantic love

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Summary Our current knowledge of the neurobiology of romantic love remains scanty. In view of the complexity of a sentiment like love, it would not be surprising that a diversity of biochemical mechanisms could be involved in the mood changes of the initial stage of a romance. In the present study, we have examined whether the early romantic phase of a loving relationship could be associated with alterations in circulating levels of neurotrophins (NTs). Plasma levels of NGF, BDNF, NT-3 and NT-4 were measured in a total of 58 subjects who had recently fallen in love and compared with those of two control groups, consisting of subjects who were either single or were already engaged in a long-lasting relationship. NGF level was significantly higher ($p < 0.001$) in the subjects in love [mean (SEM): 227 (14) pg/ml] than in either the subjects with a long-lasting relationship [123 (10) pg/ml] or the subjects with no relationship [149 (12) pg/ml]. Notably, there was also a significant positive correlation between levels of NGF and the intensity of romantic love as assessed with the passionate love scale ($r = 0.34; p = 0.007$). No differences in the concentrations of other NTs were detected. In 39 subjects in love who—after 12–24 months—maintained the same relationship but were no longer in the same mental state to which they had referred during the initial evaluation, plasma NGF levels decreased and became indistinguishable from those of the control groups. Taken together, these findings suggest that some behavioural and/or psychological features associated with falling in love could be related to raised NGF levels in the bloodstream.

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1. Introduction

Intense romantic love, a complex sentiment typically directed toward a single person, is one of the...
most overwhelming of all affective states and has been the inspiration for some of the greatest achievements of mankind. As it typically involves emotional, cognitive, behavioural and erotic components, the functions of romantic love appear not to be limited to generate offsprings, but also to promote in individuals a stable emotional environment as well as to arise pleasant and safe feelings of happiness and sex arousal (Fisher, 1998; Gonzaga et al., 2001). The process of romantic love in humans begins with falling in love, a subjective experience characterized by intense focused attention on a preferred individual, obsessive thinking about him or her, emotional dependency on and craving for emotional union with this beloved, euphoria and increased energy (Tennov, 1979).

Despite the importance that love assumes, even if transiently, in the lives of most, our current knowledge of the neural substrates and neurochemical systems involved in arousing and sustaining romantic love is still scanty. In any case, preliminary evidence seems to suggest that the neurochemical systems involved in forming romantic bonds may also be powerful stimulators of anxiety (Carter, 1998). To explain this seeming paradox, it has been postulated that human beings are anxious until a social bond is achieved and, once it happens, it is likely, although not necessary, that anxiety will be replaced by positive feelings of integration, stability, and pleasure (Kendrick, 2004). Accordingly, some authors have proposed that falling in love could be regarded as a basic emotion like anxiety or fear, due to activation of the amygdala and related circuits and neurotransmitters (Bartels and Zeki, 2000; LeDoux, 2000). Additionally, the universality, euphoria, and focused attention of early romantic love suggest that reward and motivation systems in the human brain could likely play a role (Aron et al., 2005). It is also feasible that the formation of a romantic bond may induce the synthesis and release of biochemical mediators, which remain to be identified, that may promote positive emotions, pleasant affective states and a reduced perception of anxious feelings (Esch and Stefano, 2005).

To date, the neurochemical bases of love in humans have only been studied in one investigation, which found that the intrusive thoughts of the early romantic phase of a falling in love could be due to a reduced functionality of the serotonin transporter (Marazziti et al., 1999). In any case, in view of the complexity of a sentiment like love, it would not be surprising that a diversity of biological mechanisms could be involved in the mood changes of the initial stage of a romance. It seems therefore reasonable that, apart from conventional monoaminergic systems, many other modulators, including hormones and neurotrophins may be implicated in the altered mental state associated with falling in love. Consistently, a previous report has indicated that to fall in love provokes transient hormonal changes which seem to show a gender-related specificity (Marazziti and Canale, 2004).

At this time, however, no study has examined whether the early romantic phase of a loving relation is associated with alterations in circulating levels of neurotrophins (NTs). The family of NTs in mammals includes nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), and neurotrophins 3 (NT-3) and 4 (NT-4) (Chao, 2004). These molecules, originally described as key regulators of synaptic plasticity and neural survival during development and at adulthood (Pardridge, 2002), have been increasingly recognized as potential mediators of anxiety, emotions and behavioural modifications (Aloe et al., 1994; Alleva and Santucci, 2001; Hadjiconstantinou et al., 2001; Branci et al., 2004). Given these considerations, it seemed of interest to evaluate the levels of NTs in a cohort of subjects who had recently fallen in love. These subjects were compared with two control groups consisting of individuals who were either single or were already engaged in a long-lasting relationship.

2. Methods

From August 2002 to December 2003, potential participants required to be ‘truly, deeply and madly in love’ were recruited by word of mouth and with flyers seeking individuals who were recently fallen in love. According to previous methodology (Marazziti and Canale, 2004), only subjects whose relationships have begun within the previous 6 months were considered eligible for study participation. In addition, all individuals were ascertained to spend at least 4 h a day in thinking about the romantic partner. Study subjects were required to be medically healthy. Furthermore, heavy cigarettes smokers and high-risk HIV individuals were excluded from the present investigation.

All eligible subjects were first screened by a detailed psychiatric interview and through a series of rating scales that included the Beck Depression Inventory (BDI), the State-Trait Anxiety Inventory (STAI), and the Yale-Brown Obsessive-Compulsive Rating Scale (Y-BOCS). Axis-I and axis-II disorders, axis-I diagnosis of first degree relatives and psychotropic medication intake led to immediate exclusion from the study. In addition, only subjects
who did not show an abnormal scoring at psychometric instruments were considered for participation. The cutoff points used for defining abnormal scores were in accordance with previous studies (O’Leary et al., 2000; Peres et al., 2001; Nutt et al., 2003; Wang et al., 2005). Specifically, all study participants showed BDI scores < 16, STAI scores < 46, and Y-BOCS scores lower than or equal to 7.

After these exclusions, potential participants underwent a second-line assessment. During this phase, one of the authors (E.E.) orally interviewed all individuals in a semistructured format to establish the duration, intensity, and range of his or her feelings of romantic love. At this stage, all eligible subjects were required to fill the short hand version of the passionate love scale (PLS) (Hatfield and Sprecher, 1986), thought to be a reliable means of quantifying romantic love (Bartels and Zeki, 2000). The short version of the PLS consists of 15 items (examples: ‘I would feel deep despair if — left me’; ‘For me, — is the perfect romantic partner’), each of which can be rated from 1 (‘not at all true’) to 9 (‘definitely true’) resulting in a final score ranging from 15 to 135. Subjects with a PLS score less or equal than 85 points (i.e. ‘occasional burst of passion’) at the time of first assessment were to be withdrawn from the study. At the end of these procedures, a total of 58 subjects (37 females and 21 males; mean age: 24.4 ± 3.8 years, range 18–31 years) fulfilled all the inclusion criteria and were therefore enrolled in the investigation (Group 1: ‘Subjects in love’).

For control purposes, two distinct comparison groups were selected. The first control group consisted of 58 subjects (38 females and 20 males, mean age: 26.7 ± 3.6 years, range 19–33 years) who were already engaged in a long-lasting relationship (mean duration: 49 ± 19 months) (Group 2: ‘Long-lasting relationship’). The second control group comprised a total of 58 subjects (36 females and 22 males, mean age: 26.8 ± 3.7 years, range 19–34 years) with no romantic relationship (Group 3: ‘Singles’). Both the comparison groups consisted of subjects belonging to the same environment and with similar education level of subjects in love. Similar to Group 1, all control subjects were ascertained to be free of any psychiatric illness and psychotropic medication use. All study subjects (with the exception of 17 singles) regularly indulged in a normal sexual activity as assessed by self-report questionnaires.

Participants had Italian nationality. The study protocol complied with the Declaration of Helsinki and followed the guidelines of our internal review board. All study participants provided their written informed consent.

In all study subjects, blood samples were taken in EDTA-containing tubes after a 14 h overnight fast for NTs quantification. In female participants, samples were drawn in the early follicular phase of the menstrual cycle (i.e. between the third and the fifth day of the menses). Blood samples were centrifuged at 1000g for 30 min, and immediately divided into aliquots. Plasma specimens were then frozen and stored at −20°C until analysis, which was performed in the next few days after the sampling. Plasma levels of NGF, BDNF, NT-3 and NT-4 were determined using commercially available sandwich enzyme-linked immunosorbent assays (Promega, Madison, WI, USA), as described previously (Noga et al., 2001; Kimata, 2003).

All biochemical determinations were done in duplicate and the results were averaged. Since laboratory personnel were blinded to the participants’ status, any possible measurement error was likely to be non-differential.

The data are expressed as the mean ± SEM. Data were checked for normal distribution using the Kolmogorov-Smirnov statistics. All variables had a Gaussian distribution, and parametric analyses were thus exploited. Differences in the levels of NTs among the three groups were determined by one-way ANOVA followed by Newman-Keuls multiple-comparison post-hoc test. Categorical data were analyzed by the χ² test. Correlations among the study variables were tested by the Pearson’s correlation coefficient. Multiple linear regression analysis was performed to evaluate the independence of the association between NGF levels and PLS scores among subjects in love. In this analysis, the PLS score was considered as the dependent variable, while age, gender, psychometric scores and NTs levels were entered into the model as predictors or covariates. All calculations were generated with the use of the MedCalc (MedCalc, Mariakerke, Belgium) and SPSS 11.0 (SPSS, Inc., Chicago, IL, USA) statistical packages. The level of significance was set as p < 0.05.

3. Results

The three study groups were well matched with regard to gender (χ² = 0.149, p = 0.92). However, subjects in love were significantly younger than either the subjects with a long-lasting relationship or the singles (p < 0.001 and 0.01, respectively).

As expected (Chaudhuri et al., 2005), we found no evidence for age and gender differences in
Table 1 presents the plasma NTs concentrations observed in the three study groups. As assessed by one-way ANOVA, circulating NGF levels were significantly different across the three study groups \((p < 0.001)\). Specifically, \(p\) values for the Newman–Keuls multiple comparison posthoc test indicated that NGF levels were significantly higher in the subjects in love [mean (SEM): 227 (14) pg/ml] than in either the subjects with a long-lasting relationship [123 (10) pg/ml, \(p < 0.001\)] or the subjects with no relationship [149 (12) pg/ml, \(p < 0.001\)]. On the other hand, the concentrations of BDNF, NT-3 and NT-4 did not differ among the groups \((p > 0.05\) for all, ANOVA), and were similar to those previously reported by other studies for healthy subjects \((\text{Noga et al., 2001; Kimata, 2003})\).

NGF levels were not correlated with the BDI, STAI or Y-BOCS scores neither in the entire study cohort nor in the three subjects groups (data not shown). However, among subjects in love, a significant inverse relationship was seen between levels of BDNF and Y-BOCS scores \((r = -0.28; p = 0.04)\), while NT-3 levels were negatively correlated with BDI scores \((r = -0.27; p = 0.05)\). No additional correlation between NTs levels and psychometric indexes was seen. Similarly, scores at PLS did not show any significant association with the BDI, STAI or Y-BOCS scores.

The length of the relationship and the time spent in thinking about the romantic partner did not affect the levels of NTs (data not shown). We then examined the relationship between circulating NTs and scores at the passionate love scale. The mean scoring at the PLS in subjects in love was 107.1 ± 14.9. Notably, in this group of subjects, a significant positive correlation was found between plasma NGF levels and the intensity of the romantic relationship as assessed by the PLS \((r = 0.34; p = 0.007, \text{Fig. 1})\). However, no such correlation was found between PLS and other NTs (data not shown). To determine the extent of confounding by other factors on the association between NGF concentrations and PLS, we performed multiple linear regression analysis with a model adjusting for age, gender, scores at the psychometric instruments and levels of NTs. When these predictors were entered into the multivariate model, we found that only NGF levels were independently associated with PLS scores \((\beta = 0.365, \text{standard error} = 0.016, p = 0.009)\).

In 39 subjects in love (out of the originally recruited 58) who—after 12–24 months—maintained the same relationship but were no longer in the same mental state to which they had referred during the first evaluation, plasma NTs levels were retested. Additionally, they were requested to fill the PLS for a second time to investigate whether there was a decrease in their feelings of love at this later stage. The results of this analysis showed that, albeit maintaining their relationship, PLS scores in these subjects significantly declined from 107.1 ± 14.9 to 94.2 ± 9.4 (paired Student’s \(t\) test, \(p < 0.001\)). Additionally, NGF concentrations demonstrated a significant decrease of 45.1\% (from 227 (14) pg/ml to 125 (9) pg/ml, \(p < 0.001\)) and thus became indistinguishable from those of the two comparison groups (all \(p\) values < 0.05). No significant correlation was longer evident between PLS scores and NGF levels during this second assessment \((r = 0.07; p = 0.67)\).
Levels of other NTs at this stage were similar to those observed during the first assessment and did not correlate with PLS scores. Neither the levels of NGF nor the intensity of romantic love at the first assessment were predictive in terms of romantic attachment during this second evaluation. In addition, the PLS scores at the first assessment were not predictive of NGF levels after 12-24 months (data not shown).

4. Discussion

Although the current knowledge of the neurobiological substrates of love in humans remains meagre, studies of pair bonding in sheeps and voles have reported a recurrent association between high levels of activity in the hypothalamic pituitary adrenal (HPA) axis and the subsequent expression of social behaviours and attachments (Carter et al., 1995; DeVries et al., 1995; Aragona and Wang, 2004). On the other hand, the formation of stable pair bonding has been shown to result in a reduced HPA system activation (Kendrik, 2004), perhaps accounting for health benefits that are usually attributed to loving relationships (Esch and Stefano, 2005). Accordingly, central neuropeptides, and especially oxytocin and vasopressin, which have been heavily implicated in increasing positive social behaviours in animal studies (Winslow et al., 1993; Williams et al., 1994; Insel et al., 1998; Young et al., 1998), may be also central players in the regulation of the HPA axis (Engelmann et al., 2004). It seems therefore reasonable to hypothesize that neurochemical mediators capable of modulating HPA reactivity can also be involved in the formation of social bonds.

This is, to our knowledge, the first study investigating the peripheral levels of neurotrophins in subjects in love. The main finding of our study is that levels of NGF, but not of other NTs, are significantly elevated in the early phase of a romantic love. Notably, we have also demonstrated that, at the beginning of a romance, subjects in love show a significant positive correlation between levels of NGF and the intensity of romantic feelings as assessed with the 15-item PLS. Although the mechanisms behind this selective increase of NGF remain to be determined, our data suggest that raised NGF levels when falling in love could be related to specific emotions typically associated with intense early-stage romantic love, such as emotional dependency and/or euphoria.

Nerve growth factor is a neurotrophic peptide that has been originally described as a key regulator in the survival and maturation of neurons in the peripheral and central nervous system (Levi-Montalcini et al., 1996). In addition to its neurotrophic activity, in vivo experiments have shown that NGF has the capacity to increase circulating concentrations of adrenocorticotropic and corticosterone, thus inducing activation of the HPA axis (Otten et al., 1979; Angelucci, 1994). In addition, in vitro experiments have indicated that NGF is able to induce an increase of both release and content of hypothalamic vasopressin (Scaccianoce et al., 1993), a neuropeptide which plays a pivotal role in the formation of social bonding (Winslow et al., 1993; Kendrik, 2004). Given these considerations, it would be tempting to speculate that NGF could play a role in the molecular mechanisms of human romantic love by acting as a fine modulator of distinct endocrine functions.

Alternatively, another possibility to explain our results may be that NGF plasma concentrations in subjects in love would be raised secondarily in a stress-dependent manner. Hence, it should be noted that several studies have indicated that the level of NGF in the bloodstream increases following stressful events and anxiety-associated behaviour (Aloe et al., 1994; Hadjiconstantinou et al., 2001; Branchi et al., 2004). Thus, it would be plausible to hypothesize that NGF elevation observed in our subjects in love would simply reflect the stressful conditions and/or arousal associated with the beginning of a social bond, which may in turn be useful to overcome neophobia. We believe, however, that our present results are not suggestive of such an explanation. Indeed, no significant correlation was found between NGF and anxiety and/or depression scores in subjects in love, suggesting that anxiety per se could not account for the specific increase of NGF observed in these subjects. This was further strengthened by the results of multivariate regression analysis, which showed that the association between NGF levels and PLS scores was not weakened by the potential confounding effect of psychometric indexes.

It is also important to note that when we assessed the NGF levels for a second time (12-24 months later) in the 39 subjects (out of the original group of 58 participants) who maintained the same relationship but were no longer in the same altered mental state, we found no significant difference in the circulating concentrations of this neurotrophin as compared with those measured in the two comparison groups. Additionally, the correlation between NGF levels and PLS scores was no longer evident during this second assessment. Our present findings have striking parallels in the work of Marazziti and Canale, who previously showed that...
the hormonal changes associated with falling in love are reversible, state-dependent and probably related to some unique physical and/or psychological aspects typical of the early stage of a love relationship (Marazziti and Canale, 2004). Altogether, our results and those of Marazziti and Canale strongly indicate that in humans the neurochemical bases of early-stage romantic love may be substantially different from those of longer-term romantic relationships.

Several important limitations of the present study merit consideration. First, one potential caveat of our investigation is represented by the criteria used for selecting individuals who had fallen in love. Since no definite indication is currently available in the field, we decided to use previously reported criteria (Marazziti and Canale, 2004) in order to maximize reproducibility and ensure validity of our results. In any case, an important point that should be kept in mind when interpreting our results regards the estimated temporal course of romantic love. It was indeed previously suggested that the altered mental state associated with falling in love seems to have a precise time course, with an average duration between 18 and 36 months (Tennov, 1979; Marazziti et al., 1999; Marazziti and Canale, 2004). However, our data on a significant reduction of PLS scores—paralleled by NGF decrease—12/24 months after the initial assessment seem to suggest that this time course may be even shorter, at least in some subjects. In the light of these results, we believe that at present no definite conclusions can be drawn on when 'the early-intense phase' of a romance can be considered extinguished. Future ad hoc investigations are needed to shed more light on this important issue. A second limitation of our study consists in its relatively small sample size, which was mainly due to our strict exclusion criteria. It should also be noted that, as our sample consisted mainly of female subjects, we cannot exclude the possibility of a gender bias. In any case, because the association between the intensity of romantic love and NGF levels persisted even after adjustment for potential confounders (including gender), we can reasonably rule out a major impact of the participants' demographic characteristics on our results. Another important limitation was that our data rely entirely on single biochemical measurements of plasma neurotrophins. More research employing serial sampling is therefore necessary to better elucidate the correct chronology of long-term neurotrophins modifications in subjects in love. This is particularly important especially in the light of previous evidence showing a high stability of repetitive measurements of NGF in healthy human volunteers (Lang et al., 2003). Fourth, we recognize that sampling blood after a 14 h overnight fast could have represented a stressful condition, which may have at least in part confounded circulating NGF levels. As a final caveat, our report comprised exclusively Caucasian white subjects of Italian descent. Although romantic love is widely believed to represent a cross-culturally universal phenomenon (Jankowiak and Fischer, 1992), replication of our findings in distinct ethnic populations would be desirable.

In summary, our data demonstrated for the first time that circulating levels of NGF, but not of other NTs, are elevated among subjects in love, suggesting an important role for this molecule in the 'social chemistry' of human beings. The specificity of NGF increase during early-stage love, which was independent from anxiety and/or depression scores as well as from other neurotrophins, seems to suggest that this neurokine could be especially involved in the formation of novel bonds, whereas it does not appear to play a major role in their maintenance. Although no psychophysiological explanation can be directly inferred from our observational study, we speculate that NGF could exert this effect by acting as a modulator of diverse endocrine functions, including HPA axis activation.

Given the complexity of the sentiment of romantic love, and its capacity to exhilarate, arouse, disturb, and influence so profoundly our behaviour, further investigations on the neurochemistry and neuroendocrinology of this unique emotional state are warranted.

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