Plasma oxytocin distributions in a large cohort of women and men and their gender-specific associations with anxiety

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Summary Research has consistently addressed the relations between plasma oxytocin (OT) — a nonapeptide implicated in mammalian social bonding — and psychological distress, but the direction of the association remains unclear. Utilizing the largest sample of plasma OT to date \((N=473)\), the current study had two goals. First, we described the distributions of plasma OT in women and men, and second, we examined whether the relations between OT and two types of anxiety — trait and attachment anxiety — are moderated by gender. Results indicated that OT values \(M=375.78\ pg/ml, SD=264.03, range=51.40–2752.30\) clustered around the mean with a long right tail, indicating trend toward high values. In most participants \((N=323)\), OT was measured again six months after initial assessment and OT levels were highly stable within individuals. After removing outliers \(2.5\ SD\) above the mean \((\geq988\ pg/ml\) for men and \(\geq988\ pg/ml\) for women), men showed significantly higher mean OT than women \((\text{men: } 327.13\ pg/ml, SD=164.43; \text{men: } 399.91, SD=183.65; t=2.57, p=.01)\). Gender was found to moderate the relations between OT and anxiety. Trait anxiety was lower among men with higher OT but no such links emerged for women, supporting the hypothesized anxiolytic effects of OT in males only. Furthermore, women with extreme values \((\geq988\ pg/ml)\) had three times the probability of being classified as highly anxious \((\text{STAI-T} \geq 45)\). Higher OT in women correlated with greater attachment anxiety, but no such relationships were found for men. Results are consistent with models on the differential associations between the neurobiology of attachment and the experience of anxiety in women and men.

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1. Introduction

Oxytocin (OT), a nine-amino acid peptide synthesized in the hypothalamus, has been implicated in mammalian attachment and social adaptation. OT is produced in the PVN and SON nuclei of the hypothalamus, from where it is released into distinct brain regions as well as into the posterior pituitary, which is the major source of OT in the blood (Gimpl and Fahrenholz, 2001). Research in animal models has shown that brain OT is involved in the regulation of multiple socially oriented behaviors, such as mating, pair-bonding, social memory, partner recognition, and stress and anxiety (Neumann, 2008). OT is also produced in the body and OT receptors are distributed along the gastrointestinal tract, heart, testes, uterus, corpus luteum, placenta and amnion, and are also present in the kidney, pancreas, thymus, and in adipocytes (Kiss and Mikkelsen, 2005). However, whether these peripheral sources of OT have implications for human social behavior and the experience of stress and anxiety is largely unknown (Churchland and Winkielman, 2012).

Although several researchers have suggested that the expression of central and peripheral OT is coordinated (Carter et al., 2007; Ross and Young, 2009), this issue is matter of continuous debate (Meyer-Lindenberg et al., 2012). While no study has conclusively demonstrated clear links between central and peripheral OT activity in humans, some findings may point in this direction (Weisman et al., 2012). First, studies in humans and non-human primates found correlations between peripheral OT and maternal behavior, particularly maternal touch (Maestripieri et al., 2009; Feldman et al., 2010) and these findings are consistent with those reported in rodents, which show associations between maternal touch and brain OT (Meany, 2001). In addition, risk alleles on the OXTR and CD38 genes, an ectoenzyme that mediates the release of OT from hypothalamic neurons to the bloodstream through the mobilization of calcium, were found to be associated with lower levels of plasma OT (Feldman et al., 2012). These findings accord with the hypothesis that central and peripheral OT levels are inter-related. Because human research is unable to assess OT at the brain neurochemical level, research is generally limited to peripheral measures, which have been consistently correlated with affiliation-related behaviors and cognitions, psychological stress, and social salience, similar to the associations reported between such behaviors and brain OT in other mammals (Meyer-Lindenberg et al., 2012).

A growing body of research has addressed the associations between plasma OT and a range of socially related processes in humans and animals (Heinrichs et al., 2009; McCall and Singer, 2012). The earlier studies struggled to demonstrate that plasma OT provides a valid measure that is meaningfully correlated with attachment-related processes in humans (see Feldman, 2012, for review), and can be reliably measured by ELISA and RIA methods (Carter et al., 2007; Strathearn et al., 2009). Yet, most studies used relatively small samples (Churchland and Winkielman, 2012) and we are aware of no large cohorts that detailed the distributions of plasma OT in the normative population that can provide baseline values for research on pathological conditions. An additional controversial issue is the associations between plasma OT and anxiety. OT has been repeatedly correlated with measures of stress and anxiety with inconclusive results; whereas some studies consider OT as a neuroendocrine marker of stress (Taylor et al., 2006, 2010), others emphasize its anxiolytic effects (Heinrichs et al., 2003; Light et al., 2005; Gordon et al., 2008; Ditzen et al., 2009).

Several studies suggested that OT may serve as an indicator of stress, particularly anxiety experienced in the interpersonal domain (Turner et al., 1999; Marazziti et al., 2006). For example, socially isolated female prairie wolves had more OT-immune-reactive cells in the hypothalamic PVN, and greater circulating OT concentrations 10 min after the manipulation (Grippo et al., 2007). Similarly, correlations between women’s plasma OT and relationship distress have been reported in several studies (e.g., Taylor et al., 2006, 2010; Tabak et al., 2011). However, whereas most of these studies included women only, studies enrolling both women and men showed correlations with relationship anxiety only for women. For instance, Taylor et al. (2010) found that OT correlated with relational distress in women but not men.

Feldman et al. (2011) reported associations between higher OT with parenting stress and attachment anxiety only among women.

Paradoxically, among men, an inverse pattern was found between OT and general anxiety. For example, negative correlations were found between plasma OT and trait anxiety in a sample of ninety men (Opacka-Juffry and Mohiyeddini, 2012). An imaging study including intranasal administration of OT to male participants reported reduction in amygdala activations and decreased connectivity between amygdala and brainstem regions involved in fear manifestation (Kirsch et al., 2005). These findings are consistent with those reported in animal models on the links between OT and sedation, relaxation, and reduced fearfulness (Neumann, 2008), and with human research showing correlations between OT with low anxiety and lower psychological distress (Heinrichs et al., 2009).

The aforementioned inconsistent findings may suggest that gender plays an important role in shaping the relationship between OT and anxiety. According to Taylor et al. (2010), responses to stress are gender-specific. Men use “flight or fight” behaviors, while women activate attachment-related “tend and befriend” strategies for the regulation of stress, and thus, the links between the neurobiology of attachment and the feeling of anxiety are stronger for women than men. Similarly, it has been suggested that the development of the OT system is sensitive to gender effects (Gimpl and Fahrenholz, 2001), that early rearing experiences affect the oxytocinergic system in sexually dimorphic manner (Carter, 2007; Carter et al., 2009), and that both acute and chronic OT manipulations in animals bred for high and low anxiety produce different effects in males and females (Slattery and Neumann, 2010). Yet, the gender specific associations between OT and anxiety have not been fully explored in humans.

In light of the above, the current study had two goals. First, we wished to describe the distribution characteristics of plasma OT in the largest cohort of healthy females and males reported to date. Second, we investigated whether gender moderates the relations between plasma OT and two types of anxiety — trait anxiety, representing the individual’s general level of stress, and attachment anxiety — stress specific to close relationships. Our hypothesis was that the
relationship between anxiety and OT would be moderated by gender. Attachment-related anxiety was expected to be associated with higher OT in women, but not in men. On the other hand, we examined whether OT would be associated with lower stress and anxiety in men.

2. Methods

2.1. Participants

The study included 473 participants who had their blood drawn and analyzed for OT levels. All subjects were recruited between the years 2005 and 2010 as part of a series of studies concerning the role of OT in human social behavior. Recruitment was made either through advertisements posted in the university and surrounding area or advertisements in public online portals. All procedures were carried out with the adequate understanding and written consent of the subjects.

All participants self-reported of being healthy, with no history of chronic mental or physical illness, medication intake, or any drug or alcohol abuse. Participants were with at least a high-school education, of Israeli-Jewish ethnicity, and were considered middle class SES. We collected information on women’s menstrual cycle, hormonal contraception intake, and breastfeeding status. When samples included breastfeeding mothers, OT was not sampled during the half an hour prior to or following nursing. We found no differences in baseline OT between breastfeeding or non-breastfeeding mothers or differences related to contraception intake or menstrual cycle, and all women were pooled together for the current analysis.

More than half of the participants (58.5%) were females. Of females, 72.6% were mothers and 27.4% were singles. Of males, 58.7% were fathers and 41.3% were singles. Altogether, 66.8% (316) of the entire samples were parents and 33.2% (157) were singles. Mean age of the entire sample was 26.95 years (SD = 4.39), with no age difference between genders (male: M = 27.50 years, SD = 5.36; female: M = 26.66, SD = 4.25, N5). Of these individuals, 323 had a second blood draw approximately six months after the first assessment.

2.2. Procedure

Participants arrived at the lab or met at their house between 1300 h and 1900 h. Participants were first debriefed about the study and signed informed consent, then asked to complete two anxiety questionnaires, and finally, blood was drawn by authorized nurse. Blood was collected during the above mentioned time-window to control for diurnal variation in OT, consistent with previous research (Amico et al., 1989; Gordon et al., 2010; Feldman et al., 2010).

2.3. Anxiety questionnaires

2.3.1. State-Trait Anxiety Inventory (STAI; Spielberger et al., 1970)

This well-validated 40-item scale yields separate scales that measure stable individual differences in anxiety (trait) and momentary stress (state).

2.3.2. Experience in Close Relationships (ECR; Brennan et al., 1998)

This well-validated 36-item scale measures attachment-related tendencies. The instrument yields two scores—attachment anxiety and attachment avoidance.

2.4. Plasma OT collection and determination

Blood for OT was drawn from antecubital veins into a 9 ml chilled vacutainer tubes containing lithium heparin that was supplemented with 400 KIU of Trasylol (Trasylol—Bayer, Leverkusen, Germany) per 1 ml blood. Blood samples were kept ice-chilled for a maximum time of up to 2 h before being centrifuged at 4 °C at 1000 for 15 min. Supernatants were collected and stored at —80 °C until assayed.

Determination of OT was performed by using a 96-plate commercial OT-ELISA kit (Assay-Design, MI, USA), consistent with previous research (e.g., Carter et al., 2007; Gordon et al., 2008; Taylor et al., 2010). The immunoassay for the determination of un-extracted plasma OT was found to be sensitive and reliable in previous studies (Kramer et al., 2004; Carter et al., 2007), although the kit’s manufacturer recommend that extraction will be made prior to EIA analysis. When compared with high pressure liquid chromatography (HPLC), EIA was found to be highly specific for un-extracted plasma OT (Carter et al., 2007). Samples were diluted (1:5) in the assay buffer and further treated according to kit’s instructions. Dilution has already shown to give results within the linear portion of the standard curve (Carter et al., 2007; Gouin et al., 2010). Measurements were performed in duplicates.

At the final step, the optical density of the samples and standards were measured with a wavelength of 405 and of 590 for additional correction. Sample concentrations were calculated by MatLab-7 (MathWorks, Natick, MA) according to the kit’s relevant standard curve of 15—1000 pM. The kit’s detection limit is 11.7—1000 pM. For each plate a separate standard curve was constructed. Samples were run in several batches on different occasions and the inter- and intra-assay coefficients of variation of all batches were less than 12.4% and 14.5%, respectively.

2.5. Statistical analysis

Non-parametric plasma OT distributions of women and men were compared using independent Mann—Whitney U-analysis with OT as the dependent variable. Further independent nonparametric median test was executed. Following outliers removal, distributions were log-normal and compared again with independent t-test analysis. Further analyses were conducted on the sample without the outliers.

Utilizing the log-normal distribution, associations between plasma OT and subscales of the STAI and ECR questionnaires were examined for men and women separately as well as for the entire sample using Pearson’s correlations. Chi square analysis was conducted to compare the prevalence of extremely high (STAI-T ≥ 45) versus typical scores of trait anxiety within two groups of OT values — the normally distributed values of OT and the extreme high outlier values, which were defined, consistent with prior research, as 2.5 SD higher than the mean.
Finally, two separate hierarchical regression analyses were performed in order to test the moderating role of gender on the relations between OT and trait and attachment anxiety. Predictors of trait and attachment anxiety were entered in three steps. Gender was entered in the first step, plasma OT in the second step, and an interaction of gender and plasma OT was entered in the third step. The significant interaction was followed up with tests of slopes, which assess whether the slope depicting the relationship between plasma OT and trait anxiety and the relationship between plasma OT and attachment anxiety was significantly different from zero in men and women (Aiken and West, 1991).

3. Results

3.1. Plasma OT distribution

For the entire sample, mean plasma OT was 375.78 pg/ml (SD = 264.03) with a wide range (51.40—2752.30). OT distributions clustered around the center with a long right tail (kurtosis: 21.06, $SE = 0.22$; skewness: 3.55, $SE = 0.11$). In cases where OT was re-assessed six months after initial assessment ($n = 323$), high individual stability was found for the entire sample (regardless of gender) with Pearson’s correlations ranging from $r = .75$ to $r = .92$. To examine longitudinal relationships between anxiety at T1 and OT at T2 for the sub-samples, we computed partial correlations between trait and attachment anxiety at T1 and OT at T2 while controlling for OT at T1 separately for women and men. The only significant partial correlations was between attachment anxiety at T1 and OT at T2 in women, $r = .18$, $p < .05$.

To compare the female and male distributions, we used independent Mann–Whitney $U$-analysis with OT as the dependent variable. The two distributions showed a significant difference. Mean ranks for men and women were 256.25 and 223.38, respectively; Mann–Whitney $U = 23373$, $Z = -2.57$, $p = .01$ two-tailed, suggesting that the two distributions are different (men: $M = 399.78$, $SD = 279.24$, median = 342.60, min = 51.40, max = 2752.30, range = 2700.90; women: $M = 358.80$, $SD = 251.85$, median = 289.15, min = 55.40, max = 2231.41, range = 2176.01). In addition, independent nonparametric median test turned significant ($statistic = 7.45$, $p = .006$), with men showing higher median OT than women. Plasma OT was unrelated to demographic variables, including age, education, height, weight, smoking, time since last meal, menstrual cycle phase, and contraceptive intake. Fig. 1 presents the distributions of plasma OT values for women and men.

Once outliers greater than 2.5 $SD$ above the means ($\geq 1098 \text{ pg/ml for men and } \geq 988 \text{ for women}$) were excluded ($N = 14$; 9 women, 5 men) parameters improved substantially ($M = 343.01 \text{ pg/ml, } SD = 170.72$, range 51.40—1075.23; kurtosis: 1.96, $SE = 0.22$; skewness: 1.25, $SE = 0.11$) and the distribution was log-normal. At this point, a significant difference between men and women was found in mean plasma OT levels (women: 327.13 pg/ml, $SD = 164.43$; men: 399.91, $SD = 183.65$), $t(458) = 2.57$, $p = .01$.

3.2. Correlations between OT and anxiety

Trait and state anxiety did not differ across genders (trait — women: 35.31, $SD = 9.35$; men: 35.78, $SD = 9.52$; state — women: 32.98, $SD = 8.58$; men: 32.38, $SD = 8.34$), $t(446) = .52$, $p > .05$ and $t(348) = .67$, $p > .05$, respectively. However, consistent with previous research, women and men showed significant differences in the ECR, with women scoring higher in attachment anxiety and men in attachment avoidance (anxiety — women: 3.10, $SD = 97$, men: 2.85, $SD = .96$; avoidance: women: 2.89, $SD = .87$, men: 3.32, $SD = .97$), $t(361) = 2.52$, $p = .012$ and $t(361) = 4.42$, $p < .001$, respectively.

Plasma OT was significantly correlated with trait anxiety in men, $r = -.26$, $p < .001$, but not in women, $r = .10$, $p > .05$ (Fig. 2). We conducted further analysis in order to examine whether highly anxious women tend to show extreme OT values (above 2.5 $SD$), STAI-T scores of >40 are considered to index high anxiety (Frasure-Smith et al., 1995) and we used a more stringent criteria of STAI-T $\geq$ 45 to assess women scoring above 1 $SD$ in the current sample. Women were thus divided into highly anxious (STAI-T $\geq$ 45) and non-anxious groups. Results indicated that two-thirds of the women with OT $\geq$ 988 pg/ml (above 2.5 $SD$ of the mean) are in the high-anxiety category, as compared to 20% in the women in the low-anxiety group, $\chi^2(1) = 11.27$, $p = .001$, $\eta^2 = .20$. Such difference was not evident among men. $\chi^2(1) = 0.07$, $p > .05$.

With regards to attachment anxiety, OT was negatively correlated with attachment anxiety in men: $r = -.15$, $p = .05$, whereas a positive correlation was found in women, $r = .15$, $p < .05$. No general or gender-specific associations were found between OT and state anxiety or attachment avoidance.

3.3. Gender moderates the relationship between OT and anxiety

Two hierarchical regression models predicting trait or attachment anxiety from gender, OT, and their interaction were computed. With regards to trait anxiety, the model as a whole was significant ($R^2$ total = .033, $F(3,446) = 5.04$, $p = .002$). Whereas gender or OT did not have a unique contribution to the prediction of trait anxiety, their interaction showed a significant independent effect ($\beta = .16$, $R^2$ Change = .027, $F$ Change(1,443) = 12.15, $p = .001$). Simple slope analysis (Aiken and West, 1991) revealed that the interaction was significant for men, slope ($\beta = -.26$,
Figure 2   Scatter plots (with regression lines) of the relation between plasma oxytocin and trait- and attachment-anxiety among women (left panels) and men (right panels).

\[ \frac{t = 3.62}{p < .001} \text{, but not for women, slope } (\beta) = .08, \quad t = 1.29, \quad p < .05 \text{ (ns).} \]

As to attachment anxiety, the model as a whole was again significant (\(R^2\) total = .036, \(F(3,358) = 4.46, p = .004\)). Gender had an independent contribution to the prediction of attachment anxiety (\(\beta = .13, R^2 \text{ Change } = .01, F \text{ Change}(1,357) = 5.86, p = .016\)), as women reported higher attachment anxiety than men, but not OT. Similar to the findings for trait anxiety, the interaction of OT and gender uniquely predicted attachment anxiety above and beyond gender and OT (\(\beta = .15, R^2 \text{ Change } = .020, F \text{ Change}(1,355) = 7.43, p = .007\)). Simple slope analysis revealed that the interaction was significant both for men, slope (\(\beta\)) = -.15, \(t = -1.92, p = .05\), and women, slope (\(\beta\)) = .15, \(t = 1.93, p < .05\).

4. Discussion

Results of the current study, including the largest human sample of plasma OT reported to date, demonstrate that plasma OT levels in the general population are not distributed normally and tend to be skewed to the right, pointing to a tendency toward higher levels compared to the mean. The distributions of women and men were significantly different from each other as evaluated by non-parametric statistics. After the removal of outliers, men showed higher mean levels then women, results which may be counter-intuitive. Findings also indicate that plasma OT is reliably measured and individually stable when tested within a six-month period. Finally, our data indicate that gender moderates the relations between plasma OT and trait and attachment anxiety and this moderating effect may provide an overall framework for the consistently controversial results regarding the associations between peripheral OT and measures of psychological distress in humans.

Different gender-related associations between OT and anxiety emerged for the experience of anxiety in close relationships and the disposition of anxiety as a general trait. Women who reported higher attachment anxiety had higher OT values and these findings are consistent with previous reports (e.g., Taylor et al., 2006, 2010). On the other hand, men with higher OT reported lower trait anxiety, whereas the link between anxiety and OT in women was observed only in the very high values, not in the general population. The negative associations found between OT and trait anxiety in men is consistent with a recent study (Opacka-Juffry and Mohiyeddini, 2012), which showed similar correlation among ninety healthy male volunteers and concluded that OT may serve an anxiolytic function in men. In contrast, highly anxious women tended to display extreme values of OT, suggesting that extreme anxiety may be linked with over-activation of the oxytocinergic system in women. Such over-activation may have resulted from an early childhood stress or trauma which could have led to hyper-reactivity of the system in an attempt to self-soothe and was then stabilized.
through feed-forward mechanisms. Porges and Carter (2011) suggest that the development of the OT system, which is formed by means of bio-behavioral interactions between the infant and the caregiving environment, is shaped through feed-forward mechanisms that continuously monitor the organism’s sense of safety in its current context. It is thus possible that early childhood trauma may be related to increased activations of the OT system in women, particularly since the development of the oxytocinergic system in females is sensitive to affiliation processes. Yet, much further research is required to assess whether the very high values of OT and anxiety are indeed related to childhood trauma or extreme stress in women as opposed to men.

Evolutionary perspectives may provide an explanation for the gender differences between OT and attachment anxiety. Due to their primary role in infant care and survival, bonding processes in women involve normative stress and preoccupations with infant safety and well-being (Leckman et al., 2004). Similarly, both animal and human studies show increased amygdala activations to the attachment target in mothers but not in fathers and suggest that stress and hyper-vigilance is a necessary by-product of becoming a mother and adaptation to the maternal role (Oxley and Fleming, 2000; Atzil et al., 2011, 2012). Possibly, for women, attachment-related processes, which are supported by the OT system, naturally involve high anxiety for the maintenance of the relationship, the well-being of the attachment partner, and the proper provision of adequate care, whereas such anxiety is not a component of typical bonding processes in males. Furthermore, perspectives which suggest that psychopathology represents a distortion of processes that initially had a sound evolutionarily based goal (Leckman and Mayes, 1999), may view the link between OT and attachment anxiety in women as an intensification of a process that is initially adaptive from an evolutionary perspective. The finding that attachment anxiety in women was the only form of anxiety that predicted OT levels six months later, above and beyond concurrent OT, point to the close associations between anxiety in attachment relationships and peripheral OT in women and may be consistent with the feed-forward mechanisms that support the OT system proposed by Porges and Carter (2011).

Limitations of the study relate to the fact that we relied on self-report instruments to measure anxiety and did not include more objective measures, such as cortisol levels or autonomic response. Nonetheless, the two measures of anxiety used here are considered highly reliable and have been extensively studied in human research for several decades. A second limitation concerns the relatively weak correlations found between plasma OT and measures of anxiety. Extant research in both animals and humans has shown correlations between exogenous administration of OT and reduced stress responses, attesting to the links between OT and stress in mammals (Bartz et al., 2011; Meyer-Lindenberg et al., 2012). In the present study, we did not use OT manipulations to increase the effects and only assessed baseline levels. Additionally, our sample includes only healthy young adults without any known pathology, and thus the range of values is somewhat restricted as compared to more pathological samples. It should also be noted that elevations in plasma OT do not always signal relationship distress (e.g., Feldman et al., 2007; Gordon et al., 2008) and multiple psychological processes can lead to a similar biological outcome (Churchland and Winkielman, 2012). Unlike research in animal models, a very tight fit between the human behavioral repertoire and specific neuroendocrine processes is not often found, particularly when testing very large samples, and more research is typically needed to tease apart specific conditions, subgroups, cultural backgrounds, socioeconomic contexts, or variability in personal experience that are related to variability within the general sample and their specific associations with modulations in neuroendocrine processes. The magnitude of the correlations between OT and anxiety reported here is similar to the magnitude of associations between OT and other behaviors, personality dimensions, and cognitions in other human studies and suggest that further specification is needed. Although the link between central and peripheral OT is not yet fully understood, and demonstrating such coordination was not the goal of our study, cumulative human evidence points to comparable processes in humans and other mammals with regards to the associations between OT and stress, and this consistency may be of scientific value.

Future research is needed to further understand the genetic, brain, and immunological relationships between endogenous OT and stress and anxiety in humans. Such research may lead to the development of more specific biomarkers that can serve as indices of typical versus atypical anxiety in women and men, particularly following stressful or traumatic experiences, and may ultimately result in the formation of more specific pharmacological or behavioral interventions.

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Conflict of interest

Drs. Weisman, Zagoory-Sharon, Schneiderman, Gordon and Feldman have no conflict of interest to disclose.

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