

Cumulative risk on the oxytocin receptor gene (*OXTR*) underpins empathic communication difficulties at the first stages of romantic love

Inna Schneiderman, Yaniv Kanat-Maymon, Richard P. Ebstein, and Ruth Feldman

Empathic communication between couples plays an important role in relationship quality and individual well-being and research has pointed to the role of oxytocin in providing the neurobiological substrate for pair-bonding and empathy. Here, we examined links between genetic variability on the oxytocin receptor gene (*OXTR*) and empathic behaviour at the initiation of romantic love. Allelic variations on five *OXTR* single nucleotide polymorphisms (SNPs) previously associated with susceptibility to disorders of social functioning were genotyped in 120 new lovers: *OXTR*rs13316193, rs2254298, rs1042778, rs2268494 and rs2268490. Cumulative genetic risk was computed by summing risk alleles on each SNP. Couples were observed in support-giving interaction and behaviour was coded for empathic communication, including affective congruence, maintaining focus on partner, acknowledging partner's distress, reciprocal exchange and non-verbal empathy. Hierarchical linear modelling indicated that individuals with high *OXTR* risk exhibited difficulties in empathic communication. *OXTR* risk predicted empathic difficulties above and beyond the couple level, relationship duration, and anxiety and depressive symptoms. Findings underscore the involvement of oxytocin in empathic behaviour during the early stages of social affiliation, and suggest the utility of cumulative risk and plasticity indices on the *OXTR* as potential biomarkers for research on disorders of social dysfunction and the neurobiology of empathy.

Keywords: oxytocin; *OXTR*; empathy; romantic relationships; bonding; genetic risk

INTRODUCTION

Extant evidence suggests that intimate relationships exert both beneficial and harmful effects on the individual's physical and psychological health (Cohen and Wills, 1985; Enns *et al.*, 2002; Anderson and Saunders, 2003). Communication patterns within romantic relationships are central predictors of adults' well-being and life satisfaction (Burman and Margolin, 1992; Gottman and Levenson, 1992), and research has demonstrated associations between positive communication between couples and marital satisfaction. On the other hand, communication difficulties often precede the onset of marital distress (Markman, 1981) and serve as better predictors of daily marital dissatisfaction as compared to problems in other domains (Jacobson and Moore, 1981). Observational studies have further confirmed that interaction patterns between couples are reliable predictors of change in marital stability (Gottman and Levenson, 1992). In particular, studies employing direct observations of dyadic behaviour indicated that empathic communication patterns serve as important building blocks of the couple's relationship, whereas empathic difficulties may play a causal role in relationship decline and the development of psychopathology (Gottman and Levenson, 1992; Lyons-Ruth, 2008). As relational patterns tend to persist over time and relational difficulties often escalate into behavioural cycles that may lead to separation or divorce, assessing the couple's behaviour at the first months of pair-bonding and identifying biomarkers that may underpin empathic communication difficulties during this period is of empirical and clinical relevance.

The major neuroendocrine system mediating bond formation is the oxytocin (OT) system (Carter, 1998). Research in both human and non-human mammals has implicated OT, a neuropeptide synthesized primarily in the paraventricular and supraoptic nuclei of the

hypothalamus, in social cognition, social behaviour and affiliation (Hollander *et al.*, 2007; Acevedo *et al.*, 2011; Nagasawa *et al.*, 2012). Furthermore, disruptions to OT are found in disorders involving severe dysfunction to social communication, including autism, depression and schizophrenia (Gregory *et al.*, 2009; Souza *et al.*, 2010; Apter-Levi *et al.*, 2013; Mah *et al.*, 2013). Animal studies indicate that OT receptor (*OXTR*) distributions across multiple areas of the brain mediate reproductive and sexual behaviour, parental care, social memory and attachment (Insel and Hulihan, 1995; Champagne *et al.*, 2001; Burri *et al.*, 2008; Feifel *et al.*, 2012). In addition, OT has shown to support processes of pair-bonding in humans and mammals (Bales *et al.*, 2007; Feldman *et al.*, 2012). OT is essential for the formation and regulation of pair-bonding behaviour in monogamous prairie voles (*Microtus ochrogaster*), especially in females (Insel and Hulihan, 1995; Young and Wang, 2004); central OT administration facilitates partner preference in female prairie voles (Williams *et al.*, 1994); and an OT antagonist prevents pair-bond formation (Insel and Hulihan, 1995). In humans, plasma OT levels show a substantial increase during the first year of romantic relationships in both men and women as compared to romantically unattached individuals (Schneiderman *et al.*, 2012), and peripheral OT is linked with affiliation, emotional support and positive communication between couples (Grewen, 2005; Gonzaga *et al.*, 2006; Holt-Lunstad *et al.*, 2008). Imaging studies of romantically attached individuals showed neural activations in regions associated with pair-bonding in monogamous prairie voles (Acevedo *et al.*, 2011), and intranasal OT administration increased positive communication between couples (Ditzen *et al.*, 2009). Furthermore, intranasal OT administration was found to enhance emotional empathy and positive communication and to modulate amygdala activity (Ditzen *et al.*, 2008; Singer *et al.*, 2008; Bartz *et al.*, 2010; Hurlemann *et al.*, 2010). Finally, Walum *et al.* (2012) found associations between allelic variations on the *OXTR* gene and self-reported bonding behaviour.

Although social behaviour is a complex multi-dimensional phenotype, twin and family studies suggest moderate heritability of social traits, including empathy, aggression and prosocial behaviour (Ebstein

Received 5 February 2013; Revised 9 August 2013; Accepted 18 August 2013

Advance Access publication 22 August 2013

The study was supported by the German-Israeli Foundation (#1114-101.4/2010) and the Irving B. Harris Foundation, Israel Center for Excellence (ICORE) to Ruth Feldman.

Correspondence should be addressed to Ruth Feldman, PhD, Department of Psychology and the Gonda Brain Sciences Center, Bar-Ilan University, Ramat-Gan 52900, Israel. E-mail: feldman@mail.biu.ac.il

et al., 2010). As OT is highly conserved across mammalian evolution (Insel and Young, 2000), it is possible that *OXTR* gene polymorphisms may underpin individual differences in social behaviour and empathy. In humans, the *OXTR* gene is localized as a single copy on chromosome 3, contains three introns and four exons (Inoue *et al.*, 1994), and codes for the OT receptor (*OXTR*) which belongs to the class I G protein-coupled receptor family (Gimpl and Fahrenholz, 2001). Several single nucleotide polymorphisms (SNPs) on the *OXTR* gene have been associated with prosocial behaviour and bonding as well as with greater susceptibility to psychopathology (Lerer *et al.*, 2007; Israel *et al.*, 2009; Wu *et al.*, 2012; Feldman *et al.*, 2012, 2013). In particular, the SNP rs13316193 C allele has been associated with empathy (Wu *et al.*, 2012), whereas the T allele has been linked to decreased expression of OT receptors in the brain, depressive mood and greater risk for Autism Spectrum Disorder (ASD) (Lerer *et al.*, 2007; Kawamura *et al.*, 2010; Tansey *et al.*, 2010). The G allele of the *OXTR* rs2254298 has been related to greater risk for autism, depression and separation anxiety (Jacob *et al.*, 2007; Lerer *et al.*, 2007; Costa *et al.*, 2009) and with amygdala volume decrease in both Caucasian and Asian populations (Inoue *et al.*, 2010; Furman *et al.*, 2011). The T allele of the *OXTR* rs2268490 and the G allele of the *OXTR* rs1042778 were each implicated in prosocial decision making (Israel *et al.*, 2009), and carriers of the rs1042778 G allele exhibited more parenting behaviour and had higher plasma OT levels than T carriers (Feldman *et al.*, 2012). The A allele of the SNP rs2268494 was associated with risk for ASD (Lerer *et al.*, 2007). Finally, the SNP rs53576 has been associated with autism, empathy and prosocial behaviour (Wu *et al.*, 2005; Rodrigues *et al.*, 2009; Kogan *et al.*, 2011). Overall, these data provide evidence for the involvement of *OXTR* variants in bond formation and empathic behaviour in humans.

In this study, we chose a cumulative risk approach to study risk on the *OXTR* by combining multiple SNPs into a single index. Our assumption was that as these *OXTR* variants are individually associated with prosocial traits, affiliative behaviours and psychopathology of social dysfunction, a cumulative risk score may provide an integrative measure that can be more predictive of social outcomes than each SNP alone. Using a cumulative risk score on the *OXTR*, we recently found that mothers' and fathers' cumulative *OXTR* risk predicted children's OT production and social reciprocity with their best friends (Feldman *et al.*, 2013), pointing to the utility of testing cumulative risk on the *OXTR*. This approach is consistent with current models on the combined contribution of risk and plasticity alleles in shaping psychopathology and social adaptation (Belsky *et al.*, 2009).

The overall goal of this study was to test the relations between *OXTR* polymorphisms and empathic communication between couples during a support-giving interaction that required empathy to the partner's distress (Collins and Feeney, 2000). As support in social relationships is an important contributor to well-being and relationship satisfaction (Bowlby, 1969; Feeney and Lemay, 2012) and empathic support is among the central emotional capital of intimate bonds (Feeney and Lemay, 2012), empathic support-giving behaviour was expected to index an important social attribute of the individual and to serve as a central marker of the relationship. Periods of bond formation involve adaptation of the OT system and are associated with increase in peripheral OT production (Feldman, 2012), and we thus expected *OXTR* risk to predict empathic difficulties at the first 3 months of a romantic relationship. Five *OXTR* variants that have been linked with social behaviour and pathology of social functioning were tested: rs13316193, rs2254298, rs1042778, rs2268494 and rs2268490. It was expected that greater number of risk alleles on these SNPs would predict empathic difficulties at the first stages of romantic love in a dose-response manner.

METHODS

Participants

Participants included 120 young adults (60 couples) who began a romantic relationship in the past 3 months. Men were on average 25.03 years (s.d. = 8.78) and women's age averaged 22.84 (s.d. = 4.50). All participants were Caucasians, healthy, and completed at least 12 years of education.

Exclusion criteria included couples who were in a romantic relationship <2 weeks or more than 4 months, used medication for a physical or psychiatric condition, or reported not being generally healthy. Participants were recruited through ads in several universities located in central Israel, and as participants were all college students their age range was 18–35.

Procedure

Experiments were conducted in a comfortable laboratory during the mid-afternoon hours (4:00–7:00 PM). After receiving a brief explanation on the experimental procedure and signing an informed consent, participants completed self-report measures that assessed a range of demographic and health variables (e.g. weight, height, smoking, medication). Next, mouthwashes of DNA were collected. Following these activities, participants engaged in a *social support interaction* paradigm. Partners were instructed to describe to each other a situation that caused them personal distress but was not related to the romantic relationships (e.g. work problems, family problems) and take turns within the paradigm for approximately 7 min each. Order of speaker–listener was decided by the couple. Discussions were videotaped and analysed offline using the Coding Interactive Behavior Manual (CIB) (Feldman, 1998). Each couple received 70 USD for participation. The research was approved by the University Institutional Review Board and all participants signed an informed consent.

Genotyping

Genotyping of the *OXTR* SNPs rs1042778, rs2268494, rs13316193, rs2254298 and rs2268490 was done as previously described (Lerer *et al.*, 2007; Feldman *et al.*, 2012). DNA was extracted from 20 ml of mouthwash samples using the Master Pure kit (Epicentre, Madison WI). Genotyping of the *OXTR* SNPs was performed using the SNaPshot Method (Applied Biosystems, Foster City, CA, USA) as previously described (Lerer *et al.*, 2007; Feldman *et al.*, 2012). All PCR reactions and High Resolution Melt (HRM) analysis were performed on a Rotor-Gene 3000 (Corbett Life Science, Australia), using the following primers that produced a 162 bp amplicon: F5'GGT GCACAGACCACTTAGCA'3; R5'TCGGAAGAGAGGAAAGCAA'3. PCR reaction conditions were as follows: activating enzyme step at 95.0°C for 15 min, 45 cycles of denaturation at 95.0°C for 5 s, reannealing at 60°C for 15 s and extension at 72°C for 10 s. The reaction proceeded to a hold at 40°C for 2 min, a second hold at 82°C for 2 min and then the melt procedure ramped from 82 to 90°C raising by 0.1°C every 3 s where fluorescence was acquired. HRM distinguished between the three genotypes (AA, AG, GG) and the method was verified by comparison of a portion of HRM results to those obtained by genotyping the same samples using the SNaPshot procedure (Applied Biosystems) described above. All genotype frequencies of the *OXTR* SNPs were in Hardy–Weinberg equilibrium.

Cumulative susceptibility to social and communicative difficulties was calculated by summing the number of SNPs on which the individual was homozygote for the risk allele. These included the *OXTR* rs1042778 TT allele, the *OXTR*rs2268494 AA allele, the *OXTR* rs13316193 TT allele, the *OXTR* rs2254298 GG allele and the *OXTR*rs2268490 TT allele. Thus, for each individual, the *OXTR* risk

score could range from 0 (no risk alleles) to 5 (risk factors on all five investigated *OXTR* SNPs). Descriptive statistics for the *OXTR* distribution indicate that the distribution was approximately normal as both skewness of -0.09 (s.e. = 0.22) and kurtosis of -0.24 (s.e. = 0.44) did not exceed twice their standard errors. No outliers were detected.

Behavioural coding

Support-giving interactions were coded with the CIB (Feldman, 1998), adult version, by trained coders who were blind to genetic or any other information about the participants. The CIB is a well-validated rating system of dyadic interactions with versions for coding social interactions in infancy, childhood, adolescence and adulthood. The CIB has shown adequate psychometric properties across multiple attachments in health and psychopathology, including romantic attachment (Feldman, 2012; Feldman et al., 2012; Schneiderman et al., 2012).

The adult-adult version of the CIB included 33 scales: 28 are identical scales that are coded independently for each partner (e.g. attention to partner) and five scales are coded for the couple as a whole and address dyadic measures (e.g. dyadic reciprocity). Each scale is rated on a Likert scale of 1 = low to 5 = high. Consistent with our previous research the *communicative empathy* construct was averaged from several scales coded for each partner and included the following codes: affective congruence, emotional flow and harmony, degree of emotional coherence and complexity, nonverbal signs of empathy particularly the provision of affectionate touch, attention and acknowledgement of partner's distress and communications, and maintaining focus on other's distress (Cronbach's $\alpha = 0.71$).

Self-report instruments

Depression was measured by the Beck Depression Inventory (BDI), a well-validated instrument for the assessment of depressive symptoms (Beck, 1978), whereas *Anxiety* was assessed by the Trait Anxiety Inventory (STAI-T) (Spielberger et al., 1983). The standardized BDI and STAI-T scores were summed to create a composite of the individual's *emotional distress*.

Statistical analysis

Associations among the research variables and between romantic partners were assessed with the Pearson correlation coefficient. As participants were nested within dyads, multi-level modelling (MLM) was used to examine the effects of cumulative *OXTR* risk on empathic communication while controlling for dyadic dependency. All *P*-values were two-tailed. Data were analysed using SPSS software for Windows, version 19 (SPSS Inc., Chicago, IL, USA).

RESULTS

As a first step, we computed correlations between all study variables for women and men. Table 1 presents the inter-correlations, means and standard deviations for all study variables. As seen, empathic communication difficulties showed a significant negative correlation with the degree of genetic risk on the *OXTR* for both men and women, indicating that the greater the cumulative risk on the *OXTR* the less the individual engaged in empathic support-giving behaviour. Genetic risk was negatively associated with emotional distress for men, but not for women.

Next, we examined whether genetic risk on the *OXTR* predicts variability in empathic communication while controlling for gender, relationship duration and emotional distress. The structure of the data was nested because data were collected from both romantic partners during the conversation. Indeed, as seen in Table 1, significant positive

Table 1 Inter-correlations, means, and standard deviations of study variables for women and men

| Measures | 1 | 2 | 3 | <i>M</i> | s.d. |
|-----------------------------|--------|--------|--------|----------|------|
| 1. <i>OXTR</i> genetic risk | 0.16 | -0.27* | -0.20* | 2.02 | 0.74 |
| 2. Emotional distress | 0.05 | -0.05 | 0.04 | 0.56 | 0.42 |
| 3. Empathic communication | -0.30* | 0.17 | 0.88** | 4.11 | 0.57 |
| <i>M</i> | 1.98 | 0.61 | 4.01 | | |
| s.d. | 0.87 | 0.73 | 0.58 | | |

Men above the diagonal, women below the diagonal and dyads on the diagonal. * $P < 0.05$, ** $P < 0.01$.

correlation was found between the partners' scores on empathic communication, indicating dyadic dependency. An MLM analysis (Campbell and Kashy, 2002; Kenny et al., 2006) was used to model the dyadic dependency (i.e. non-independence) and to test whether the individual's *OXTR* risk uniquely predicts his or her own empathic communication above and beyond the dyadic level. Prior to computing the model we checked for order effect (who spoke first) and as no main or interactive effects for order were found and thus order was not included in the final model. We used the MIXED procedure in SPSS (Heck et al., 2010) with restricted likelihood estimation to estimate the coefficients (Kenny et al., 2006). Gender, relationship duration and emotional distress were included as important correlates in order to control for their potential variation. All the predictors were centred around the grand mean. Interactions between *OXTR* risk and gender were also included to examine whether the association between genetic risk and empathic communication vary across genders.

We started by running a null model for empathic communication to assess whether our data met the condition justifying dyadic analysis, that is, the data show systematic between-dyad variance in the dependent variable. Results suggest that this condition has been satisfied (Wald $Z = 5.05$, $df = 59$, $P < .001$). Estimating the null model also produces information necessary for computing intraclass correlation coefficient (ICC) that indicates the proportion of between-dyad variance in empathic communication (cf. Kenny et al., 2006). The results of this analysis indicate that 88% of the variance in empathic communication lies between dyads. We also assessed the ICC for the rest of dyadic predictors and found that 17% of the variance in *OXTR* risk lies between dyads and 1% of the variance in emotional distress lies between dyads.

Next, we ran a model which included all the predictors. Comparing the estimates of the residual variance produced in the null model and the current model allowed for the computation of the variance explained (R^2). Results indicated the current model accounted for 12% of the variance. Table 2 provides coefficients for the study variables predicting empathic communication. As seen, genetic risk on the *OXTR* uniquely predicted the individual's own empathic communication, indicating that the higher the cumulative risk on the *OXTR* the less the individual engaged in empathic support-giving behaviour during the interaction. Importantly, the *OXTR* risk by gender interaction term was not statistically significant. This indicates that the association between genetic risk and empathic communication does not vary between men and women. Gender was found to be a significant predictor of empathic communication with men being more empathic to the distress of their partners during the first months of a romantic relationship. Relationship duration and emotional distress were not significantly predictive of empathic communication in men or women.

Table 2 MLM unstandardized and standardized coefficients predicting empathic communication from gender, relationship duration, emotional distress, cumulative *OXTR* risk and cumulative *OXTR* genetic risk by gender

| | <i>b</i> | s.e. | 95% CI | β |
|---|----------|------|----------------|---------|
| Gender | -0.05* | 0.02 | -0.10 to -0.01 | -0.09 |
| Relationship duration | 0.07 | 0.06 | -0.09 to 0.22 | 0.05 |
| Emotional distress | 0.01 | 0.01 | -0.01 to 0.01 | 0.06 |
| Cumulative <i>OXTR</i> risk | -0.09* | 0.03 | -0.18 to -0.01 | -0.14 |
| Cumulative <i>OXTR</i> risk \times gender | -0.05 | 0.03 | -0.13 to 0.02 | -0.06 |

Table 3 Risk on each SNP of the *OXTR* and empathic communication between romantic partners

| SNPs | Low risk mean (s.d.) | High risk mean (s.d.) | <i>F</i> | <i>P</i> -value |
|------------|----------------------|-----------------------|----------|-----------------|
| rs1042778 | 4.10 (0.55) | 3.70 (0.72) | 4.91 | 0.03 |
| rs2268494 | 4.21 (0.54) | 4.03 (0.58) | 1.67 | N.S. |
| rs13316193 | 4.09 (0.50) | 4.04 (0.65) | 0.25 | N.S. |
| rs2254298 | 4.15 (0.59) | 4.01 (0.58) | 1.6 | N.S. |
| rs2268490 | 4.06 (0.58) | 4.00 (0.55) | 0.01 | N.S. |

Finally, to supplement our cumulative risk approach and the MLM analysis, Table 3 presents means, s.d., and *F*-values of the differences between high- and low *OXTR* risk for each SNP separately. As seen, although the results for each *OXTR* SNP show higher empathic communication among individuals with the low risk allele, only the *OXTR*rs1042778 independently differentiated those with high and low risk alleles. These data support our cumulative risk approach and demonstrate the utility of combining several theoretically meaningful SNPs into a single composite.

DISCUSSION

Empathic communication between couples provides the foundation for growth-promoting intimate relationships, whereas empathic difficulties often result in relationship deterioration and emotional distress (Anderson and Saunders, 2003). Yet, whereas much current research points to the interactions between genetic markers and human social behaviour, most studies on the genetic basis of empathy utilized self-reports or experimental paradigms and no study, to our knowledge, employed direct observations of empathic behaviour within real relationships (Feldman, 2012). Results of this study are also the first to link genetic variability with observed behaviour in the context of romantic bonding. Furthermore, our findings are the first to describe associations between cumulative genetic risk on the *OXTR* and empathy. The findings indicate that risk alleles on the *OXTR* underpin difficulties in empathic communication at the early stages of romantic love, a period associated with alterations in brain activity and neurohormonal processes (Marazziti *et al.*, 1999; Bartels and Zeki, 2000; Aron, 2005; Emanuele *et al.*, 2006; Kim *et al.*, 2009). Specifically, individuals with greater cumulative risk on the *OXTR* variants rs13316193, rs2254298, rs1042778, rs2268494 and rs226849 exhibited less empathic concern to their partner's distress, showed lower affective congruence, displayed lower social reciprocity in a support-giving interaction, and persisted less in attending to their partner's communication and maintaining focus on providing support. Furthermore, the MLM analysis demonstrates that the association between *OXTR* risk and the individual's empathic behaviour is significant above and beyond the dyadic level, relationship duration and emotional distress. These data extend previous research on the involvement of the OT system in human

empathy and social affiliation and demonstrate for the first time associations between the *OXTR* genotype and communication patterns between couples.

Our results are consistent with studies reporting associations between *OXTR* variants and greater susceptibility to social deficits. Previous research has linked these *OXTR* variants with less empathic and prosocial behaviour and with conditions associated with severe social dysfunction, such as ASD and depression (Lerer *et al.*, 2007; Costa *et al.*, 2009; Israel *et al.*, 2009; Kawamura *et al.*, 2010; Wu *et al.*, 2012). It has been proposed that the social deficits in these disorders, which are particularly noted in aspects of interpersonal communication, may be mediated by individual differences in amygdala development that are related to the *OXTR* genotype (Inoue *et al.*, 2010; Meyer-Lindenberg *et al.*, 2011). Indeed, some of the *OXTR* variants studied here have been associated with amygdala volume, morphology and activity (Inoue *et al.*, 2010; Tost *et al.*, 2010; Furman *et al.*, 2011). Similarly, animal research indicates that OT shows significant binding in the amygdala (Bale *et al.*, 2001; Huber *et al.*, 2005), and OT in the medial amygdala is essential for social recognition in mice (Ferguson *et al.*, 2001). In humans, intranasal OT administration was found to reduce right amygdala responses (Domes *et al.*, 2007). Taken together, these findings underscore the links between the OT system and individual differences in interpersonal relationships as well as difficulties in social communication, possibly via the close connection between brain OT and the extended amygdala network. The present findings add the dimension of cumulative genetic risk on the *OXTR* in shaping the individual's concrete empathic behaviour during the initial stages of pair-bonding in humans.

Current conceptual models highlight the utility of assessing cumulative genetic risk as an underlying explanatory framework for the study of psychiatric disorders (Belsky *et al.*, 2009). It is important to note that there is always a challenge in how best to use all the genetic information in a set of SNPs available from the dbSNP database. Approaches include genotype and haplotype-based methods as well as principle components analysis. Genotype strategies, for example, may use a single-SNP analysis and each marker is tested individually for its contribution to the phenotype (Chapman and Wijsman, 1998; Schork *et al.*, 2000; Kaplan and Morris, 2001). On the other hand, Gauderman *et al.* (2007) has suggested a principle components analysis. There is also a joint-SNP approach using multiple SNPs simultaneously in the statistical model (Chapman *et al.*, 2003; Clayton *et al.*, 2004). In our study, which contains a relatively small sample for a genetic study, we chose to reduce the dimensionality associated with a large number of possible haplotypes and to eliminate the uncertainty connected with the need to estimate haplotypes or haplotype blocks. For these reasons, we implemented a joint-SNP analysis.

It has been suggested that allelic variations on multiple genes can mark either risk or resilience pending on the individual's rearing environment, and some allelic variations that predispose individuals to psychopathology under aversive rearing conditions may have beneficial effects in more favourable environments (Belsky *et al.*, 2009). These conceptual models may explain the mixed results reported with regards to *OXTR* variants. For instance, whereas some researchers found that the *OXTR* rs1042778 G allele is implicated in prosocial behaviour and parental care (Israel *et al.*, 2009; Feldman *et al.*, 2012), as we found here, others reported associations between rs1042778 G allele and social deficits. Similarly, culture effects have been noted with respect to the *OXTR* rs2254298 variant. Whereas in Caucasian samples the G genotype was related to unipolar depression, adult separation anxiety and higher risk for autism (Lerer *et al.*, 2007; Costa *et al.*, 2009), in Asian population the opposite A allele was associated with ASD (Wu *et al.*, 2005). In addition, some studies did not find *OXTR* gene involvement in social behaviour or in the aetiology of autism (Apicella

et al., 2010; Tansy et al., 2010). These findings underscore the need for much further research on the potential utility of assessing plasticity variants on the *OXTR* in combination with measures that provide a detailed assessment of early environmental experiences. It is important to carefully chart cultural, environmental and allelic biomarkers in order to compute comprehensive indices that can be used as markers of risk or resilience in the context of psychiatric vulnerability.

The finding that men showed greater empathy than women during a support-giving encounter was somewhat surprising. While we do not have a clear explanation for this finding, it is possible that during the first months of romantic courtship men are especially attentive to their partner's signals whereas women may be more anxious and preoccupied, which may deploy their support-giving abilities. Support for this hypothesis comes from our finding that during the first months of romantic love, women's cortisol levels show a substantial rise whereas men's cortisol levels remain unchanged. In addition, we found that during the first months of romantic love men show greater ERP response to attachment-related cues (Weisman et al., 2012). Still, much further research is required to test gender-specific patterns of communication and their biological underpinnings during the period of falling in love.

Evolutionary models suggest that periods of bond formation—in particular parental and romantic bonding—are associated with functional adaptation of brain and behaviour, share underlying bio-behavioural mechanisms, and are associated with the OT system and with a specific repertoire of bonding-related behaviour (Feldman et al., 2012). However, whereas much research explored the neurobiological mechanisms underlying parental bonding, very little research examined genetic, brain, or hormonal correlates of romantic bond-formation in humans and little, if any, research included direct observations of dyadic behaviour during the stage of falling in love. Applying an ethological approach to the study of affiliative bonds, long advocated by Lorenz (1950) and Tinbergen (1963), we suggest that observation of specific affiliative behaviour during periods when bonds are consolidated may provide an important lens to study human attachment. As suggested by the ethologists, observed patterns of behaviour may be more closely related to underlying neurobiological processes and may provide a bottom-up, behaviour-based evaluation of distress or psychopathology at an earlier stage of the relationship as compared to more formal assessments.

Limitations of the study include the omission of the *OXTR* rs53576, which has been associated with prosocial behaviour, maternal sensitivity and attachment (Bakermans-Kranenburg and Van IJzendoorn, 2008; Costa et al., 2009; Tost et al., 2010), from the panel of *OXTR* SNPs tested in this study. The rs53576 SNP was not assayed as we were unable to find appropriate primers to fit the existing SNAPSHOT panel. Similarly, although the study included healthy, functioning adults we do not have detailed information on their rearing conditions apart from general report of growing within typical rearing environments. Future studies should examine the associations between social behaviour, *OXTR* genotypes, brain structures associated with social behaviour and early environmental conditions in an attempt to create a cumulative risk index from these multiple biological and behavioural markers that can serve as an early diagnostic tool for identifying vulnerability to psychopathology at its earliest stages. Understanding the differential influence of *OXTR* genotypes on social communication behaviour via the modulation of brain development and activity may enable gene-based therapy in conditions associated with severe social dysfunctions, such as autism, post-traumatic stress disorder, or depression. Such research may provide new avenues for understanding the roles of specific allelic variations on the *OXTR* and their cumulative activity on brain, social behavioural patterns

and the development of empathy under conditions of health and psychopathology.

REFERENCES

- Acevedo, B.P., Aron, A., Fisher, H.E., Brown, L.L. (2011). Neural correlates of long-term intense romantic love. *Social Cognitive and Affective Neuroscience*, 7, 145–59.
- Anderson, D.K., Saunders, D.G. (2003). Leaving an abusive partner an empirical review of predictors, the process of leaving, and psychological well-being. *Trauma, Violence, & Abuse*, 4, 163–91.
- Apicella, C.L., Cesarini, D., Johannesson, M., et al. (2010). No association between oxytocin receptor (*OXTR*) gene polymorphisms and experimentally elicited social preferences. *PLoS One*, 5, e11153.
- Apter-Levi, Y., Feldman, M., Vakart, A., Ebstein, R.P., Feldman, R. (2013). Impact of maternal depression across the first six years of life on the child's mental health, social engagement, and empathy: the moderating role of oxytocin. *American Journal of Psychiatry*, 170(10), 1161–68.
- Aron, A. (2005). Reward, motivation, and emotion systems associated with early-stage intense romantic love. *Journal of Neurophysiology*, 94, 327–37.
- Bakermans-Kranenburg, M.J., Van IJzendoorn, M.H. (2008). Oxytocin receptor (*OXTR*) and serotonin transporter (5-HTT) genes associated with observed parenting. *Social Cognitive and Affective Neuroscience*, 3, 128–34.
- Bale, T.L., Davis, A.M., Auger, A.P., Dorsa, D.M., McCarthy, M.M. (2001). CNS region-specific oxytocin receptor expression: importance in regulation of anxiety and sex behavior. *The Journal of Neuroscience*, 21, 2546–52.
- Bales, K.L., Mason, W.A., Catana, C., Cherry, S.R., Mendoza, S.P. (2007). Neural correlates of pair-bonding in a monogamous primate. *Brain Research*, 1184, 245–53.
- Bartels, A., Zeki, S. (2000). The neural basis of romantic love. *Neuroreport*, 11, 3829–34.
- Bartz, J.A., Zaki, J., Bolger, N., et al. (2010). Oxytocin selectively improves empathic accuracy. *Psychological Science*, 21, 1426–8.
- Beck, A.T. (1978). *Beck Depression Inventory*. San Antonio, TX: Harcourt Brace Jovanovich.
- Belsky, J., Jonassaint, C., Pluess, M., Stanton, M., Brummett, B., Williams, R. (2009). Vulnerability genes or plasticity genes? *Molecular Psychiatry*, 14, 746–54.
- Bowlby, J. (1969). *Attachment and Loss: Attachment*. London: The Hogarth Press and the Institute of Psychoanalysis.
- Burman, B., Margolin, G. (1992). Analysis of the association between marital relationships and health problems: an interactional perspective. *Psychological Bulletin*, 112, 39.
- Burri, A., Heinrichs, M., Schedlowski, M., Kruger, T.H.C. (2008). The acute effects of intranasal oxytocin administration on endocrine and sexual function in males. *Psychoneuroendocrinology*, 33, 591–600.
- Campbell, L., Kashy, D.A. (2002). Estimating actor, partner, and interaction effects for dyadic data using PROC MIXED and HLM: a user-friendly guide. *Personal Relationships*, 9, 327–42.
- Carter, C.S. (1998). Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology*, 23, 779–818.
- Champagne, F., Diorio, J., Sharma, S., Meaney, M.J. (2001). Naturally occurring variations in maternal behavior in the rat are associated with differences in estrogen-inducible central oxytocin receptors. *Proceedings of the National Academy of Sciences of the United States of America*, 98, 12736–41.
- Chapman, J.M., Cooper, J.D., Todd, J.A., Clayton, D.G. (2003). Detecting disease associations due to linkage disequilibrium using haplotype tags: a class of tests and the determinants of statistical power. *Human Heredity*, 56, 18–31.
- Chapman, N.H., Wijsman, E.M. (1998). Genome screens using linkage disequilibrium tests: optimal marker characteristics and feasibility. *American Journal of Human Genetics*, 63, 1872–85.
- Clayton, D., Chapman, J., Cooper, J. (2004). Use of unphased multilocus genotype data in indirect association studies. *Genetic Epidemiology*, 27, 415–28.
- Cohen, S., Wills, T.A. (1985). Stress, social support, and the buffering hypothesis. *Psychological Bulletin*, 98, 310–57.
- Collins, N.L., Feeney, B.C. (2000). A safe haven: an attachment theory perspective on support seeking and caregiving in intimate relationships. *Journal of Personality and Social Psychology*, 78, 1053–73.
- Costa, B., Pini, S., Gabelloni, P., et al. (2009). Oxytocin receptor polymorphisms and adult attachment style in patients with depression. *Psychoneuroendocrinology*, 34, 1506–14.
- Ditzen, B., Schaefer, M., Gabriel, B., Bodenmann, G., Ehlert, U., Heinrichs, M. (2009). Intranasal oxytocin increases positive communication and reduces cortisol levels during couple conflict. *Biological Psychiatry*, 65, 728–31.
- Ditzen, B., Schmidt, S., Strauss, B., Nater, U.M., Ehlert, U., Heinrichs, M. (2008). Adult attachment and social support interact to reduce psychological but not cortisol responses to stress. *Journal of Psychosomatic Research*, 64, 479–86.
- Domes, G., Heinrichs, M., Gläscher, J., Büchel, C., Braus, D.F., Herpertz, S.C. (2007). Oxytocin attenuates amygdala responses to emotional faces regardless of valence. *Biological Psychiatry*, 62, 1187–90.
- Ebstein, R.P., Israel, S., Chew, S.H., Zhong, S., Knafo, A. (2010). Genetics of human social behavior. *Neuron*, 65, 831–44.

- Emanuele, E., Politi, P., Bianchi, M., Minoretto, P., Bertona, M., Geroldi, D. (2006). Raised plasma nerve growth factor levels associated with early-stage romantic love. *Psychoneuroendocrinology*, 31, 288–94.
- Enns, M.W., Cox, B.J., Clara, I., et al. (2002). Parental bonding and adult psychopathology: results from the US National Comorbidity Survey. *Psychological Medicine*, 32, 997–1008.
- Feeney, B.C., Lemay, E.P., Jr (2012). Surviving relationship threats: the role of emotional capital. *Personality & Social Psychology Bulletin*, 38, 1004–17.
- Feifel, D., Macdonald, K., Cobb, P., Minassian, A. (2012). Adjunctive intranasal oxytocin improves verbal memory in people with schizophrenia. *Schizophrenia Research*, 139, 207–10.
- Feldman, R. (1998). *Coding Interactive Behavior*. Unpublished manual.
- Feldman, R. (2012). Oxytocin and social affiliation in humans. *Hormones and Behavior*, 61, 380–91.
- Feldman, R., Gordon, I., Influx, M., Gutbir, T., Ebstein, R.P. (2013). Parental oxytocin and early caregiving jointly shape children's oxytocin response and social reciprocity. *Neuropsychopharmacology: Official Publication of the American College of Neuropsychopharmacology*, 38, 1154–62.
- Feldman, R., Zagoory-Sharon, O., Weisman, O., et al. (2012). Sensitive parenting is associated with plasma oxytocin and polymorphisms in the OXTR and CD38 genes. *Biological Psychiatry*, 72, 175–81.
- Ferguson, J.N., Aldag, J.M., Insel, T.R., Young, L.J. (2001). Oxytocin in the medial amygdala is essential for social recognition in the mouse. *The Journal of Neuroscience*, 21, 8278–85.
- Furman, D.J., Chen, M.C., Gotlib, I.H. (2011). Variant in oxytocin receptor gene is associated with amygdala volume. *Psychoneuroendocrinology*, 36, 891–7.
- Gauderman, W.J., Murcray, C., Gilliland, F., Conti, D.V. (2007). Testing association between disease and multiple SNPs in a candidate gene. *Genetic Epidemiology*, 31, 383–95.
- Gimpl, G., Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiological Reviews*, 81, 629–83.
- Gonzaga, G.C., Turner, R.A., Keltner, D., Campos, B., Altemus, M. (2006). Romantic love and sexual desire in close relationships. *Emotion (Washington, DC)*, 6, 163–79.
- Gottman, J.M., Levenson, R.W. (1992). Marital processes predictive of later dissolution: behavior, physiology, and health. *Journal of Personality and Social Psychology*, 63, 221–33.
- Gregory, S.G., Connelly, J.J., Towers, A.J., et al. (2009). Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. *BMC Medicine*, 7, 62.
- Green, K.M. (2005). Effects of partner support on resting oxytocin, cortisol, norepinephrine, and blood pressure before and after warm partner contact. *Psychosomatic Medicine*, 67, 531–8.
- Heck, R.H., Thomas, S.L., Tabata, L.N. (2010). *Multilevel and Longitudinal Modeling with IBM SPSS*, 1st edn. Chicago, IL: Routledge Academic.
- Hollander, E., Bartz, J., Chaplin, W., et al. (2007). Oxytocin increases retention of social cognition in autism. *Biological Psychiatry*, 61, 498–503.
- Holt-Lunstad, J., Birmingham, W.A., Light, K.C. (2008). Influence of a 'warm touch' support enhancement intervention among married couples on ambulatory blood pressure, oxytocin, alpha amylase, and cortisol. *Psychosomatic Medicine*, 70, 976–85.
- Huber, D., Veinante, P., Stoop, R. (2005). Vasopressin and oxytocin excite distinct neuronal populations in the central amygdala. *Science (New York, NY)*, 308, 245–8.
- Hurlmann, R., Patin, A., Onur, O.A., et al. (2010). Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *Journal of Neuroscience*, 30, 4999–5007.
- Inoue, H., Yamasue, H., Tochigi, M., et al. (2010). Association between the oxytocin receptor gene and amygdala volume in healthy adults. *Biological Psychiatry*, 68, 1066–72.
- Inoue, T., Kimura, T., Azuma, C., et al. (1994). Structural organization of the human oxytocin receptor gene. *The Journal of Biological Chemistry*, 269, 32451–6.
- Insel, T.R., Hulihan, T.J. (1995). A gender-specific mechanism for pair bonding: oxytocin and partner preference formation in monogamous voles. *Behavioral Neuroscience*, 109, 782–9.
- Insel, T.R., Young, L.J. (2000). Neuropeptides and the evolution of social behavior. *Current Opinion in Neurobiology*, 10, 784–9.
- Israel, S., Lerer, E., Shalev, I., et al. (2009). The oxytocin receptor (OXTR) contributes to prosocial fund allocations in the dictator game and the social value orientations task. *PLoS One*, 4, e5535.
- Jacob, S., Brune, C.W., Carter, C.S., Leventhal, B.L., Lord, C., Cook, E.H., Jr (2007). Association of the oxytocin receptor gene (OXTR) in Caucasian children and adolescents with autism. *Neuroscience Letters*, 417, 6–9.
- Jacobson, N.S., Moore, D. (1981). Spouses as observers of the events in their relationship. *Journal of Consulting and Clinical Psychology*, 49, 269–77.
- Kaplan, N., Morris, R. (2001). Prospects for association-based fine mapping of a susceptibility gene for a complex disease. *Theoretical Population Biology*, 60, 181–91.
- Kawamura, Y., Liu, X., Akiyama, T., et al. (2010). The association between oxytocin receptor gene (OXTR) polymorphisms and affective temperaments, as measured by TEMPS-A. *Journal of Affective Disorders*, 127, 31–7.
- Kenny, D.A., Kashy, D.A., Cook, W.L. (2006). *Dyadic Data Analysis*. New York, NY: Guilford Press.
- Kim, W., Kim, S., Jeong, J., et al. (2009). Temporal changes in functional magnetic resonance imaging activation of heterosexual couples for visual stimuli of loved partners. *Psychiatry Investigation*, 6, 19–25.
- Kogan, A., Saslow, L.R., Impett, E.A., Oveis, C., Keltner, D., Saturn, S.R. (2011). Thin-slicing study of the oxytocin receptor (OXTR) gene and the evaluation and expression of the prosocial disposition. *Proceedings of the National Academy of Sciences of the United States of America*, 108, 19189–92.
- Lerer, E., Levi, S., Salomon, S., Darvasi, A., Yirmiya, N., Ebstein, R.P. (2007). Association between the oxytocin receptor (OXTR) gene and autism: relationship to Vineland Adaptive Behavior Scales and cognition. *Molecular Psychiatry*, 13, 980–8.
- Lorenz, K.Z. (1950). The comparative method in studying innate behavior patterns. *Physiological Mechanisms in Animal Behavior. (Society's Symposium IV.)*. Oxford, England: Academic Press, pp. 221–68.
- Lyons-Ruth, K. (2008). Contributions of the mother-infant relationship to dissociative, borderline, and conduct symptoms in young adulthood. *Infant Mental Health Journal*, 29, 203–18.
- Mah, B.L., Van Ijzendoorn, M.H., Smith, R., Bakermans-Kranenburg, M.J. (2013). Oxytocin in postnatally depressed mothers: its influence on mood and expressed emotion. *Progress in Neuro-Psychopharmacology & Biological Psychiatry*, 40, 267–72.
- Marazziti, D., Akiskal, H.S., Rossi, A., Cassano, G.B. (1999). Alteration of the platelet serotonin transporter in romantic love. *Psychological Medicine*, 29, 741–5.
- Markman, H.J. (1981). Prediction of marital distress: a 5-year follow-up. *Journal of Consulting and Clinical Psychology*, 49, 760–2.
- Meyer-Lindenberg, A., Domes, G., Kirsch, P., Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine. *Nature Reviews. Neuroscience*, 12, 524–38.
- Nagasawa, M., Okabe, S., Mogi, K., Kikusui, T. (2012). Oxytocin and mutual communication in mother-infant bonding. *Frontiers in Human Neuroscience*, 6, 31. doi:10.3389/fnhum.2012.00031.
- Rodrigues, S.M., Saslow, L.R., Garcia, N., John, O.P., Keltner, D. (2009). Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proceedings of the National Academy of Sciences of the United States of America*, 106, 21437–41.
- Schneiderman, I., Zagoory-Sharon, O., Leckman, J.F., Feldman, R. (2012). Oxytocin during the initial stages of romantic attachment: relations to couples' interactive reciprocity. *Psychoneuroendocrinology*, 37, 1277–85.
- Schork, N.J., Nath, S.K., Fallin, D., Chakravarti, A. (2000). Linkage disequilibrium analysis of biallelic DNA markers, human quantitative trait loci, and threshold-defined case and control subjects. *American Journal of Human Genetics*, 67, 1208–18.
- Singer, T., Snozzi, R., Bird, G., et al. (2008). Effects of oxytocin and prosocial behavior on brain responses to direct and vicariously experienced pain. *Emotion (Washington, DC)*, 8, 781–91.
- Souza, R.P., De Luca, V., Meltzer, H.Y., Lieberman, J.A., Kennedy, J.L. (2010). Schizophrenia severity and clozapine treatment outcome association with oxytocinergic genes. *The International Journal of Neuropsychopharmacology*, 13, 793–8.
- Spielberger, C.D., Gorsuch, R.L., Lushene, R. (1983). *Manual for the State-Trait Anxiety Inventory*. Palo Alto, CA: Consulting Psychologists Press.
- Tansey, K.E., Brookes, K.J., Hill, M.J., et al. (2010). Oxytocin receptor (OXTR) does not play a major role in the aetiology of autism: genetic and molecular studies. *Neuroscience Letters*, 474, 163–7.
- Tinbergen, N. (1963). On aims and methods of ethology. *Zeitschrift für Tierpsychologie*, 20, 410–33.
- Tost, H., Kolachana, B., Hakimi, S., et al. (2010). A common allele in the oxytocin receptor gene (OXTR) impacts prosocial temperament and human hypothalamic-limbic structure and function. *Proceedings of the National Academy of Sciences of the United States of America*, 107, 13936–41.
- Walum, H., Lichtenstein, P., Neiderhiser, J.M., et al. (2012). Variation in the oxytocin receptor gene is associated with pair-bonding and social behavior. *Biological Psychiatry*, 71, 419–26.
- Weisman, O., Feldman, R., Goldstein, A. (2012). Parental and romantic attachment shape brain processing of infant cues. *Biological Psychology*, 89, 533–8.
- Williams, J.R., Insel, T.R., Harbaugh, C.R., Carter, C.S. (1994). Oxytocin administered centrally facilitates formation of a partner preference in female prairie voles (*Microtus ochrogaster*). *Journal of Neuroendocrinology*, 6, 247–50.
- Wu, N., Li, Z., Su, Y. (2012). The association between oxytocin receptor gene polymorphism (OXTR) and trait empathy. *Journal of Affective Disorders*, 138, 468–72.
- Wu, S., Jia, M., Ruan, Y., et al. (2005). Positive association of the oxytocin receptor gene (OXTR) with autism in the Chinese Han population. *Biological Psychiatry*, 58, 74–7.
- Young, L.J., Wang, Z. (2004). The neurobiology of pair bonding. *Nature Neuroscience*, 7, 1048–54.