HHS Public Access

Author manuscript

J Reprod Infant Psychol. Author manuscript; available in PMC 2016 February 22.

Published in final edited form as:

J Reprod Infant Psychol. 2016 ; 34(1): 77–89. doi:10.1080/02646838.2015.1072764.

Attachment and Temperament Revisited: Infant Distress, Attachment Disorganization, and the Serotonin Transporter Polymorphism

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Abstract

Objective—This study's aim was to evaluate whether infant disorganized attachment and infant proneness to distress exhibited differential relations to infant genetic factors as indexed by the serotonin transporter polymorphism.

Background—The role of the short allele of the serotonin transporter polymorphism (5-HTTLPR) in enhancing sensitivity to fearful and negative affect has been well-established (Canli & Lesch, 2007). In the current study, we used this known property of the short allele to provide a test of an important postulate of attachment theory, namely that infant attachment security or disorganization is not a function of the infant's proneness to distress.

Methods—Participants were 39 parents and infants assessed between 12 and 18 months in the Strange Situation procedure. Genotype categories for the 5-HTTLPR (and rs25531) were created by both the original and the reclassified grouping system; infant proneness to distress was assessed directly in the Strange Situation Procedure. We also assessed maternal behavior at 18 months to evaluate whether any observed genetic effect indicated a passive effect through the mother.

Results—Consistent with previous findings, the 5-HTTLPR short allele was significantly related to the infant's wariness and distress, but was not related to attachment security or attachment disorganization. In addition, maternal disrupted interaction with the infant was not related to infant genotype or infant distress.

Conclusion—Results support the concept that infant proneness to distress is associated with serotonergic factors while infant attachment security or disorganization is not a function of either 5-HTTLPR or behaviorally rated proneness to distress.

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Keywords

serotonin transporter; 5-HTTLPR; infant distress; infant attachment; infant disorganization

One long-standing debate in developmental psychology concerns how infant temperament relates to various components of infant attachment behavior. In a comprehensive literature review, Vaughn, Bost, and van IJzendoorn (2008) concluded that individual differences in attachment cannot be explained by temperamental constructs and conversely that attachment does not explain individual differences in temperament.

With the advent of molecular genetic methods, it is important to revisit this question of the relation between infant temperament and infant attachment using genetic indicators of temperament. The present study aims to test the hypothesis that infant attachment behavior is not rooted in temperamental differences and, that infant proneness to distress does reflect temperamental factors by using one genetic component of human temperament, the serotonin transporter polymorphism (5-HTTLPR), and assessing its relation to both infant attachment security and infant wariness and distress, using the standard Strange Situation Procedure (SSP, Ainsworth, Blehar, Waters, and Wall, 1978) in a longitudinal sample of families at socioeconomic risk.

Early Studies Exploring Temperament and Attachment

Belsky and Rovine (1987) initially showed that mother-reported reactive infant temperament was associated both with infant distress and with the degree of distress/proximity seeking in the SSP, but was not associated with infant attachment security. Since then, other studies have also confirmed that parental reports of child temperament are not associated with attachment security (see Vaughn et al., 2008, for a review). Barnett, Ganiban, and Cicchetti (1999), studying a high-risk sample, further extended this work and found that observed distress during the SSP was independent of infant attachment disorganization. Assessing disorganized attachment is particularly important to unravel whether the odd, out-of-context behaviors are related to temperamental or genetic diathesis to stress (see Barnett et al., 1999; Spangler & Grossmann, 1993).

The Serotonin Transporter Polymorphism as a Genetic Marker of Temperament

In humans and non-human primates, the promoter region of the serotonin transporter gene (5-HTT, SLC6A4) has a polymorphism (5-HTTLPR: 5-HTT linked polymorphic region) which affects the rate of gene transcription. The short (S) variant shows a threefold reduction in transcription compared to the long (L) variant (Lesch et al., 1996). Therefore, the S allele may account for reduced serotonin uptake in the serotonergic synapses. Presence of the S allele has been repeatedly associated with increased amygdala response to negative stimuli in adults, as well as to increased anxiety, suggesting a diathesis to negative or fearful temperament (Canli & Lesch, 2007; Hairiri & Holmes, 2006). The 5-HTTLPR has also been associated with parental reports of distress in infants (see Papageorgiou & Ronald, 2013, for a review). Thus, the 5-HTTLPR short allele has been established as one genetic factor associated with temperamental vulnerability to negative affect. Later studies indicated a functional A/G polymorphism (rs25531) within the 5-HTTLPR, therefore the distinction between L_A and L_G alleles became important, the L_G allele showed decreased transcriptional activity similar to the S allele (Hu et al., 2006; Parsey et al., 2006; Praschak-Rieder et al., 2007).

The Serotonin Transporter Polymorphism and Infant Attachment

The literature assessing the connection between 5-HTTLPR and parent-child attachment is extremely limited (see Papageorgiou & Ronald, 2013, for a review). Lakatos and her colleagues (2003) demonstrated that the 5-HTTLPR S allele was related to infants' response to novelty and anxious behavior but was *not* related to disorganized attachment. In contrast, Spangler, Johann, Ronai, and Zimmermann (2009) found that attachment disorganization and the 5-HTTLPR S allele were significantly related. Also, a gene-environment interaction indicated that this genetic association was valid only for infants of mothers exhibiting low responsiveness. Further, a study assessing electrodermal reactivity in preschool children found that during the TSST-C children with secure attachment appeared significantly less stressed if they had 5-HTTLPR L/L genotype compared to S/L or S/S genotype (Gilissen, Bakermans-Kranenburg, van IJzendoorn, & Van der Veer, 2008). However, these 5-HTTLPR findings were not replicated in other studies (Luijk et al., 2011; Pauli-Pott, Friedl, Hinney, & Bebebrand, 2009; see Cicchetti, Rogosch, & Toth, 2011 for mixed findings). Overall, the scarce literature does not provide strong evidence of a main effect of the 5-HTTLPR short allele on attachment.

Attachment and Temperament Revisited: Are Genetic Assessments of Infant Temperament Differentially Related to Distress to Separation Versus Security on Reunion?

Two studies investigated the contribution of 5-HTTLPR genotype to the prediction of the child's distress reactivity compared to the child's attachment security in infancy. Raby et al. (2012) assessed these relations at 12 and 18 months. They also assessed a complementary model in which maternal sensitive responsiveness should predict attachment security but not infant distress to separation. To index infant distress reactivity, they followed Belsky and Rovine (1987) and used the attachment sub-classifications to create two groups of children: low-distress (B_1, $B_{2,}$ and A) and high-distress (B_3, B_4, and C). They then compared genetic prediction of this grouping, presumed to index infant distress, to the more conventional grouping of infants as B (secure) versus A/C (insecure). In a longitudinal sample of 154 low-income mother-child dyads, they found that maternal responsiveness (assessed at 6 months of age) predicted infant attachment security at 12 months, while infant 5-HTTLPR predicted the infant high-distress vs low-distress grouping at 12 months. However, this pattern did not hold at 18 months. Raby and colleagues (2012) did not find any interaction effect predicting attachment security or infant distress grouping. Although these results partially support the theoretical model, the study had some limitations. First, the authors did not separate out disorganized infants, choosing to force-classify them into their best-fitting organized alternative. However, disorganized attachment patterns have been predictive of later maladaptive outcomes (van IJzendoorn, Schuengel, & Bakermans-Hranenburg, 1999), making them a group of great interest regarding the interplay of temperamental and caregiving contributions.

Roisman, Booth-Laforce, Belsky, Burt, & Groh (2013), relying on the normative-risk sample of the NICHD Study of Early Child Care and Youth Development, also used in the Luijk et al. (2011) study, did not find significant association of the 5-HTTLPR with attachment security or disorganization assessed with the SSP at 15 months. There was also no interaction effect between 5-HTTLPR and maternal sensitivity in predicting attachment security, although unexpectedly, the association between sensitivity and disorganization was marginally negative for children with the 5-HTTLPR L/L genotype and marginally positive for S allele carriers (S/L and S/S). However, they failed to replicate the finding of Raby et al. (2012) that high-distress infants, based on Belsky and Rovine's (1987) procedure, are more likely to be carriers of the 5-HTTLPR short allele, although they found a small effect in the opposite direction for White infants.

Rather than assessing infant distress directly, Raby et al. (2012) and Roisman et al. (2013) used a grouping of attachment subclassifications, as noted above, as a proxy for measuring the infant's distress reactivity. Raby et al. (2012) point out that “one important task for future research will be to replicate these findings using more direct measures of infants' distress during the strange situation” p. 1021).

The Present Study

The present study addresses these limitations. First, we evaluate the relation of the serotonin transporter polymorphism to the extent of infant disorganization, in addition to security versus insecurity. Second, we code infant distress as well as other wariness-related attachment behaviors directly in the SSP, rather than using subtypes of attachment classifications as proxies of distress. Finally, any observed genetic effect might indicate a direct effect on infant behavior or a passive effect through the mother if the mother has correlated genetic variants and a resulting propensity toward negative affect. Therefore, we also assessed maternal behavior at 18 months. The overall aim of the study was to evaluate whether infant disorganized attachment and infant proneness to distress exhibited differential relations to genetic markers of infant temperament as indexed by the serotonin transporter polymorphism.

Method

Participants

Participants were 39 mothers and their infants aged 12 to 18 months (*M* = 12.9, *SD* = .58; *M* = 18.6, *SD* = .58; 20 male). Families were participants in a longitudinal study of the influence of social risk factors on infant development and all were under federal poverty levels. Families in this study represented those families who could be relocated to contribute genetic assessments when the infants had reached 20 years of age. From an infancy cohort of 76 families, 65 infants were relocated in young adulthood, 56 participated in the follow-up study, and 39 agreed and provided adequate DNA samples. Thirty-one percent of mothers were not high school graduates, and 26% were single parents. Twenty-nine (74.4%) were Caucasian, 3 (7.7%) African American, 1 (2.6%) Hispanic, and 6 (15.4%) mixed ethnicity. The study protocol was approved by the Institutional Review Board and informed consent was obtained.

Instruments and Procedures

Assessment of serotonin transporter polymorphism—DNA samples were collected by buccal swabs; Schleicher & Schuell IsoCode ID kits were used for 6 samples that were collected by mail. DNA was isolated from buccal epithelial cells using Purgene DNA Purification kits (Gentra). Genotyping of the 5-HTTLPR and rs25531 was performed by the method of Wendland et al. (2006), using 2 independent DNA samples per subject to increase the success rate of the first genotyping round(in case of a technical problem with one sample, the second sample may still provide a clear result in the first genotyping round). Real discrepancy (such as good signal SS in one sample and good signal SL or LL in the other sample) was not observed. In case of low signal in both samples, the genotyping procedure was repeated to avoid a possible allele-dropout effect. Biallelic genotype classification using the designated functional allele S' for S and L_G alleles, was carried out as described previously (Parsey et al., 2006). Genotype frequencies did not show significant deviation from the Hardy Weinberg equilibrium in the whole group (p = .701 with 8 S/S, 18 S/L, and 13 L/L, among those 3 subjects had L_A/L_G), or in the Caucasian only sub-group (p = .832 with 6 S/S, 15 S/L, and 8 L/L, with 1 subject having L_A/L_G).

Infant Behaviors

Infant attachment—Mothers and infants were videotaped in the Strange Situation Procedure (SSP; Ainsworth et al., 1978) at 12 to 18 months of age. The SSP consists of a series of eight structured 3-minute episodes involving the baby, the mother, and a stranger. During the observation the mother leaves and rejoins the infant twice, first leaving the infant with the stranger, then leaving the infant alone. The procedure is designed to be mildly stressful in order to increase the intensity of activation of attachment behavior. The three organized attachment classifications (secure, avoidant, ambivalent) were assigned by both a computerized multivariate classification procedure developed on the original Ainsworth data (Connell, 1976; for additional details, see Lyons-Ruth, Connell, Zoll, & Stahl, 1987) and by a coder trained by M. Main. Agreement between the two sets of classifications was 86%. Agreement on disorganized categorization between two coders for 32 tapes was 83% (k = .73) and reliability of the 9-point scale for level of disorganization was r = .84. Thirty-seven infants were assessed at 18 months and 2 additional infants were assessed at 12 months. Twenty-four of those assessed at 18 months also had data at 12 months. The distribution of attachment classifications at 12 months was 46% Secure, 23% Avoidant, and 31% Disorganized; at 18 months, 29% Secure, 17% Avoidant, and 54% Disorganized. No infants displayed organized ambivalent patterns.

Infant distress and continuous attachment behaviors in the SSP were rated on seven 9-point scales developed by Ainsworth et al. (1978), including extent of distress, avoidance of mother, resistance to mother, resistance to stranger, proximity-seeking to mother, contact-maintaining with mother, and extent of exploration (Table 1). Reliabilities ranged from r = .97 to .72 (n = 12).

Family Risk Factors

Cumulative demographic risk was indexed by summing the presence of the following five characteristics: mother had no high school education, government aid recipient, no partner in home, mother under 20 at birth of first child, and more than two children under age 6.

Maternal psychosocial risk was represented by a 3-point scale where 3 = presence of documented maternal maltreatment of child and/or psychiatric hospitalization of mother; 2 = clinical level of maternal depression on the CES-D (score 16 or greater) but neither psychiatric hospitalization nor maltreatment; and 1 = absence of all of the three risk factors above. The risk distribution was as follows: maltreatment/hospitalization = 26%, depression alone = 28%, and no psychosocial risk = 46%.

Center for Epidemiological Studies Depression Scale (CES-D) is a 20-item, 60-point self-report scale widely used to measure current levels of depressive symptoms in adults. The reliability and validity of the CES-D has been well-established, with 100% sensitivity and 88% specificity in relation to clinical diagnosis using the established cut-off scores (Radloff & Locke, 1986).

Disrupted maternal communication with the infant was coded using the Atypical Maternal Behavior Instrument for Assessment and Classification (AMBIANCE; Lyons-Ruth, Bronfman, & Parsons, 1999) over all episodes of the SSP. The AMBIANCE yields a scaled score (1-7) for overall Level of Disrupted Communication which takes into account five subtypes of maternal disrupted communication: 1) affective communication errors, 2) role confusion 3) negative-intrusive behavior, 4) fearful-disoriented behavior, and 5) withdrawal. Reliability computed on fifteen tapes yielded a weighted kappa = .93. Excellent psychometric properties of the AMBIANCE have been confirmed by meta-analysis (Madigan et al., 2006).

Analytic Procedures

Only participants with genetic data were included. As recommended by Greenland & Finkle (1995), multiple imputation was used to estimate missing data on behavioral variables, using the Markov Chain Monte Carlo procedure. The rate of missing behavioral data was 13.4% (range = 0 to 33% missing). This range is well within the recommended allowances for imputation procedures. In the present study, 10 data sets were generated with excellent efficiency according to Rubin's (1987) guidelines. Genetic association testing was carried out by regression analyses using the number of S alleles or the S allele present vs absent categories.

Results

Descriptive and Control Analyses

Descriptive data for the continuous variables is shown in Table 1. Cumulative demographic risk and infant gender were unrelated both to distress to separation (see below; r = .08, n.s; t

= 1.10, n.s., respectively) and to the 5-HTTLPR genotype, $F(1,38) = .21$, n.s.; $\chi^2 = .77$, n.s. respectively. Therefore, these variables were not considered further.

Data Reduction Procedures for Infant Behavior

As expected from previous literature (Ainsworth et al., 1978), infant behaviors related to distress, contact-seeking, proximity maintaining, exploration, and resistance to the stranger during the SSP were significantly correlated with one another (12 months: *r* range = .40 to .63; 18 months: *r* range = .42 to .70) and these infant behaviors also showed significant stability from 12 to 18 months (*r* range = .60 to .70). In order to aggregate the data and reduce the number of tests, a principal components factor analysis with varimax rotation was conducted using all rating scales indexing infant behavior during the SS at both 12 and 18 months.

Total crying loaded highly (> .50) only on Factor 1, which accounted for 53.4% of the variance. Loadings for Factor 1 are shown in Table 2. This factor was clearly interpretable as the factor indexing proneness to distress, which consisted of high (>.50) loadings for greater distress, greater contact-maintaining to mother, greater resistance to the stranger, greater proximity-seeking to mother, less exploration, and less avoidance of mother at both 12 and 18 months. Factorial analyses were also performed at 12 months and 18 months separately to assess the stability of the factor structure over time and were used for further follow-up of the main analyses. The factor structures observed at 12 and 18 months were both highly similar to the combined factor structure (Table 2).

Maternal Caregiving Risk, Infant Attachment, the Serotonin Transporter Polymorphism, and Infant Proneness to Distress

As shown in Table 3, both maternal risk variables (i.e., maternal psychosocial risk and maternal disrupted communication) were unrelated to the infants' proneness to distress, $r = -.22$, n.s., $r = -.16$, n.s., respectively, and were also unrelated to infant genotype ($r = -.13$, n.s., $r = -.03$, n.s.). Finally, infant attachment security (12 and 18 months) and disorganization (12 and 18 months) were also unrelated to proneness to distress (all *p*-values between .27 and .96, ns.).Thus, the results did not support the possibility that maternal negative interaction with the infant was associated with either infant 5-HTTLPR or infant proneness to distress.

Infant Distress and the Serotonin Transporter Polymorphism

A regression analysis indicated that the number of 5-HTTLPR short alleles carried by the infant was significantly related to the factor score indexing infant proneness to distress (Table 3). The number of 5-HTTLPR short alleles was also separately related to the 12-months factor score, but not to the 18-months factor score (Table 3). To test the dominant effect of the short allele, the S/S and S/L genotypes were grouped together. Both the combined factor and the 12- and 18-months factors separately showed significant associations with genotypes containing the short allele (*p*-values < .05). Using the functional 5-HTTLPR genotype system (where the L_A/L_G is coded as a functional heterozygote) did not change these results substantially (combined factor: $p = .026$, 12-months factor: $p = .063$, and 18-months factor: $p = .037$) [1]. To address the potential problem of different ethnicities of the infants, ethnicity was included as a control variable in the regression analyses, but it did not change the final results. In addition, the genetic association between infant proneness to distress and the 5-HTTLPR short allele could be observed in the Caucasian only sub-group (p-values < .05).

Infant Attachment Security and the Serotonin Transporter Polymorphism

A second set of analyses examined whether the number of 5-HTTLPR short alleles was related to standard classifications of secure vs. insecure infant attachment security (with best-fitting classifications assigned to disorganized infants (see Raby et al., 2012) or to the extent of attachment disorganization. Neither the secure vs. insecure classification nor the extent of attachment disorganization was related to the genetic variable (Table 3).

Discussion

This study investigated whether infant disorganized attachment and infant proneness to distress exhibited differential relations to infant temperament as indexed by the serotonin transporter polymorphism. As hypothesized, the number of 5-HTTLPR short alleles carried by the infant was significantly related to the broadband factor score indexing distress, proximity seeking, contact maintaining, and reduced exploration elicited by brief separations. Neither the secure vs. insecure classification of attachment nor the extent of disorganized behavior was related to the 5-HTTLPR.

The finding that secure vs. insecure classification of attachment was not associated with the 5-HTTLPR is in agreement with earlier studies (e.g., Luijk et al., 2011; Roisman et al., 2013; see Vaughn et al., 2008, Papageorgiou & Ronald, 2013 for reviews). Importantly, these data also replicate the previous Barnett et al. (1999) finding that the extent of attachment disorganization is not related to the degree of behaviorally assessed wariness and distress and extend this finding to one genetic molecular index of fearful temperament. As discussed by Barnett et al. (1999), the disorganized attachment classification is quite heterogeneous in relation to degree of infant distress, as disorganized infants can be sub-classified as disorganized-avoidant, disorganized-resistant, and even disorganized-secure. Results suggest that this heterogeneity in distress among disorganized infants has temperamental contributions similar to those among infants with organized attachment behavior, but that temperament is not a factor that distinguishes among infants who display more or less evidence of disorganization. This lack of differentiation of more disorganized infants by the 5-HTTLPR also converges with previous findings by Lakatos et al. (2003). However, that study did not include concomitant tests of the association between 5-HTTLPR and assessments of infant distress, as done here.

These results provide further evidence of a genetic contribution from the 5-HTTLPR to infant proneness to distress and extend previous work by Raby and colleagues (2012). They explored the same research question using a less direct assessment of infant proneness to

[1]The results did not reach significance when only crying was used as an index of distress. However, 11% of the variance in 12-months crying was accounted for by the number of 5-HTTLPR short alleles, consistent with results from the broader construct used here. The number of 5-HTTLPR short alleles predicted only 2% of the variance in 18-months crying, however, suggesting that explicit crying to separation is less strongly tied to this biological substrate with increasing age.

distress. Raby and colleagues found the predicted significant association between the 5-HTTLPR short allele and distress-related attachment subtypes at 12 months but not at 18 months. However, our results were mixed in regard to age of assessment. First, direct assessments at 12 and 18 months of distress, proximity seeking, contact maintenance, reduced exploration, and resistance to the stranger were highly associated with one another and loaded on the same factor, which was in turn related to number of short alleles. The number of 5-HTTLPR short alleles was also separately related to the 12-months factor score, but not to the 18-months factor score (Table 3), similar to Raby et al. (2012). However, when the S/S and S/L genotypes were grouped together to test the dominant effect of the short allele, both the combined factor and the 12- and 18-months factors separately showed significant associations with genotypes containing the short allele (p-values $< .05$). Thus, using a dominant gene model, our findings suggest a robust effect over this age range. In addition, because the overall finding replicated when we considered the dominant effect of having any 5-HTTLPR short allele, rather than the number of short alleles, this suggests that the findings are robust across various operational definitions of the focal measures.

One potential explanation for the increased stability of the results from 12 to 18 months in the current study may be that the subclassifications of attachment do not provide as sensitive or as stable an index of infant wariness and distress as infant behaviors coded directly. Another possible explanation is that the subclassifications do not have demonstrated coder reliability and therefore may be more prone to coder error and thus less stable over time. Here, the direct assessment of proneness to distress demonstrated stability over time as well as a consistent relation to the short serotonin transporter allele.

These results are inconsistent with the Roisman et al. (2013) study showing no association between 5-HTTLPR and infant distress. One explanation for these contradictory results is that contextual risk may moderate these associations. Whereas the Roisman et al. study relied on a relatively low risk sample (e.g., mothers had on average 12-14 years of education), both Raby et al. (2012) and the current study are based on uniformly low-income samples. Therefore, there is a possibility that the similar results of both studies might represent a genetic effect elicited only under stressful environmental conditions. The few existing studies evaluating gene-environment interactions on child attachment and proneness to distress yielded mixed results (Luijk et al., 2011; Raby et al., 2012, Spangler et al., 2009). Given the modest size of our sample of families with genetic data, a gene-environment interaction effect was not assessed. Future studies are needed in high-risk samples with the power to address moderation of genetic effects.

Our findings are also consistent with heritability studies that have shown that genetic differences explain a significant amount of the variance in temperamental traits (Bokhurst et al., 2003; Roisman & Fraley, 2008). At the same time, these results add further evidence that genetic factors have a negligible effect on attachment (e.g., Bokhurst et al., 2003; O'Conner & Croft, 2001; Roisman & Fraley, 2008). A major limitation of this study is the relatively small sample size, and therefore, it is important that non-significant results be interpreted with caution as significant associations may have been undetected. However, this is partially offset by the confirmatory, rather than exploratory, nature of the analyses conducted. The function of the 5-HTTLPR short allele in relation to negative affect has already been established in other studies, and the hypotheses regarding attachment and temperament have been advanced in previous studies. However, no other study has evaluated the differential relevance of temperament to attachment disorganization in a high risk sample using both behavioral and genetic assessments. Thus, the contribution of this study is to confirm, using both molecular genetic evidence and direct assessments of infant distress, the differential correlates of disorganized attachment behavior and distress-related behavior under conditions of social risk.

Acknowledgments

Funding: We would like to thank our most important collaborators, the families who have so generously given their time to this study. This research was supported by NIMH Grant R01 MH062030 to Dr. Lyons-Ruth.

References

Ainsworth, MDS.; Blehar, MC.; Waters, E.; Wall, S. Patterns of attachment: A psychological study of the strange situation. Hillsdale, NJ: Erlbaum; 1978.

Barnett D, Ganiban J, Cicchetti D. Maltreatment, negative expressivity, and the development of Type D attachments from 12 to 24 months of age. Monographs of the Society for Research in Child Development. 1999; 64:97–118. [PubMed: 10597544]

Belsky J, Rovine M. Temperament and attachment security in the strange situation: An empirical rapprochement. Child Development. 1987; 60:787–795. [PubMed: 3608649]

Bokhorst CL, Bakermans-Kranenburg MJ, Pasco Fearon RM, van IJzendoorn MH, Fonagy P, Schuengel C. The Importance of Shared Environment in Mother–Infant Attachment Security: A Behavioral Genetic Study. Child Development. 2003; 74:1769–1782. [PubMed: 14669895]

Canli T, Lesch KP. Long story short: the serotonin transporter in emotion regulation and social cognition. Nature Neuroscience. 2007; 10:1103–1109. [PubMed: 17726476]

Connell, DB. Unpublished doctoral dissertation. Syracuse University; Syracuse, NY: 1976. Individual differences in attachment: An investigation into stability, implications, and relationships to structure of early language development.

Greenland S, Finkle WD. A critical look at methods for handling missing covariates in epidemiologic regression analyses. American Journal of Epidemiology. 1995; 142:1255–1264. [PubMed: 7503045]

Gilissen R, Bakermans-Kranenburg MJ, van IJzendoorn MH, van der Veer R. Parent–child relationship, temperament, and physiological reactions to fear-inducing film clips: Further evidence for differential susceptibility. Journal of Experimental Child Psychology. 2008; 99(3):182–195. [PubMed: 17681350]

Hairiri AR, Holmes A. Genetics of emotional regulation: the role of the serotonin transporter in neural function. Trends Cognitive Science. 2006; 4:182–191.

Hu XZ, Lipsky RH, Zhu G, Akhtar LA, Taubman J, Greenberg BD, et al. Serotonin transporter promoter gain-of-function genotypes are linked to obsessive-compulsive disorder. American Journal of Human Genetics. 2006; 78(5):815–826. [PubMed: 16642437]

Lakatos K, Nemoda Z, Birkas E, Ronai Z, Kovacs E, Ney K, et al. Association of D4 dopamine receptor gene and serotonin transporter promoter polymorphisms with infants' response to novelty. Molecular Psychiatry. 2003; 8:90–7. [PubMed: 12556912]

Luijk M, Roisman GI, Haltigan J, Tiemeier H, Booth-LaForce C, van IJzendoorn MH, Bakermans-Kranenburg MJ. Dopaminergic, serotonergic, and oxytonergic candidate genes associated with infant attachment security and disorganization? In search of main and interaction effects. Journal of Child Psychology and Psychiatry. 2011; 52:1295–1307. [PubMed: 21749372]

Lesch KP, Bengel D, Heils A, Sabol SZ, Greenberg BD, Petri S, et al. Association of anxiety-related traits with a polymorphism in the serotonin transporter gene regulatory region. Science. 1996; 274(5292):1527–1531. [PubMed: 8929413]

Lyons-Ruth, K.; Bronfman, E.; Parsons, E. Maternal frightened, frightening, or atypical behavior and disorganized infant attachment patterns. In: Vondra, J.; Barnett, D., editors. Atypical patterns of infant attachment: Theory, research and current directions Monographs of the Society for Research in Child Development. Vol. 64. 1999. p. 67-96.

Lyons-Ruth K, Connell D, Zoll D, Stahl J. Infants at social risk: Relationships among infant maltreatment, maternal behavior, and infant attachment behavior. Developmental Psychology. 1987; 23:223–232.

Madigan S, Bakermans-Kranenburg MJ, van IJzendoorn MH, Moran G, Pederson DR, Benoit D. Unresolved states of mind, anomalous parental behavior, and disorganized attachment: A review and meta-analysis of a transmission gap. Attachment and Human Development. 2006; 8:89–111. [PubMed: 16818417]

O'Conner TG, Croft MC. A twin study of attachment in preschool children. Child Development. 2001; 72:1501–1511. [PubMed: 11699684]

Papageorgiou KA, Ronald A. “He who sees things grow from the beginning will have the finest view of them” A systematic review of genetic studies on psychological traits in infancy. Neuroscience and Biobehavioral Reviews. 2013; 37:1500–1517. [PubMed: 23644044]

Parsey RV, Hastings RS, Oquendo MA, Hu X, Goldman D, Huang YY, et al. Effect of a triallelic functional polymorphism of the serotonin-transporter-linked promoter region on expression of serotonin transporter in the human brain. American Journal of Psychiatry. 2006; 163(1):48–51. [PubMed: 16390888]

Pauli-Pott U, Friedl S, Hinney A, Bebebrand J. Serotonin transporter gene polymorphism (5-HTTPLR), environmental conditions, and developing negative emotionality and fear in early childhood. Journal of Neural Transmission. 2009; 116:503–512. [PubMed: 19137235]

Praschak-Rieder N, Kennedy J, Wilson AA, Hussey D, Boovariwala A, Willeit M, et al. Novel 5-HTTLPR allele associates with higher serotonin transporter binding in the putamen: A [11C] DASB positron emission tomography study. Biological Psychiatry. 2007; 62:327–331. [PubMed: 17210141]

Raby KL, Cicchetti D, Carlson EA, Cutuli JJ, Englund MM, Egeland B. Genetic and caregiving-based contributions to infant attachment: Unique associations with distress reactivity and attachment security. Psychological Science. 2012; 23(9):1016–1023. [PubMed: 22829464]

Radloff, LS.; Locke, BZ. The community mental health assessment survey and the CES-D scale. In: Weissman, MM.; Myers, JK.; Ross, CE., editors. Community surveys of psychiatric disorders Surveys in Psycholosocial Epidemiology. Vol. 4. 1986. p. 177-189.

Roisman GI, Booth-Laforce C, Jay Belsky J, Burt KB, Groh AM. Molecular-genetic correlates of infant attachment: A cautionary tale. Attachment & Human Development. 2013; 15:384–406. [PubMed: 23421800]

Roisman GI, Fraley RC. A Behavior–Genetic Study of Parenting Quality, Infant Attachment Security, and Their Covariation in a Nationally Representative Sample. Developmental Psychology. 2008; 44:831–839. [PubMed: 18473647]

Rubin, DB. Multiple imputation for nonresponse surveys. New York: Wiley; 1987.

Spangler G, Grossmann KE. Biobehavioral organization in securely and insecurely attached infants. Child Development. 1993; 64:1439–1450. [PubMed: 8222882]

Spangler G, Johann M, Ronai Z, Zimmermann P. Genetic and environmental influence on attachment disorganization. Journal of Child Psychology and Psychiatry. 2009; 50:952–961. [PubMed: 19673052]

van IJzendoorn MH, Schuengel C, Bakermans-Kranenburg MJ. Disorganized attachment in early childhood: Meta-analysis of precursors, concomitants, and sequelae. Development and Psychopathology. 1999; 11(2):225–250. [PubMed: 16506532]

Vaughn, BE.; Bost, KK.; van IJzendoorn, MH. Attachment and temperament: Additive and interactive influences on behavior, affect, and cognition during infancy and childhood. In: Cassidy, J.; Shaver, PR., editors. Handbook of attachment: Theory, research, and clinical applications. New York, NY: Guilford Press; 2008. p. 192-216.

Wendland JR, Martin BJ, Kruse MR, Lesch KP, Murphy DL. Simultaneous genotyping of four functional loci of human SLC6A4, with a reappraisal of 5-HTTLPR and rs25531. Molecular Psychiatry. 2006; 11:224–226. [PubMed: 16402131]

Table 1

Descriptive Statistics for Continuous Study Variables

	12 mos. Mean (SD)	18 mos. Mean (SD)
Infant behavior		
Total crying	2.02(2.31)	1.97(2.07)
Contact maintenance with mother	5.28(3.00)	4.36(2.29)
Resistance to stranger	4.10(3.02)	3.67(2.63)
Resistance to mother	2.69(1.28)	2.79(1.48)
Proximity seeking to mother	6.73(2.92)	6.36(2.94)
Exploration	4.11(2.00)	4.71(1.96)
Avoidance of mother	5.07(3.21)	5.72(2.68)
Disorganized behavior score[a]	3.02(1.10)	3.63(1.16)
Family Risk Factors		
Cumulative demographic risk	1.90 (.85)	
Maternal psychosocial risk	1.79(.83)	
Level of maternal disrupted communication	4.55(1.45)	

[a]The earliest scale for disorganized attachment was a five-point scale, with four additional half-points, yielding nine scale points as used here. Scores of 4 and above yield a classification of disorganized.

Table 2

Factor Loadings

Factor 1 (Eigenvalue = 46.69)	Correlation to factor
Combined Factor 12-18 months (Eigenvalue = 46.69; Variance accounted for = 53.4%)	
Crying (12 months)	.826
Crying (18 months)	.820
Contact maintenance with mother (12 months)	.758
Contact maintenance with mother (18 months)	.831
Resistance to stranger (12 months)	.806
Resistance to stranger (18 months)	.685
Proximity seeking to mother (12 months)	.728
Proximity seeking to mother (18 months)	.804
Exploration (12 months)	-.703
Exploration (18 months)	-.797
Avoidance of mother (12 months)	-.722
Avoidance of mother (18 months)	-.677
Factor 12 months (Eigenvalue = 29.49; Variance accounted for = 65.71%)	
Crying (12 months)	.847
Contact maintenance with mother (12 months)	.820
Resistance to stranger (12 months)	.812
Proximity seeking to mother (12 months)	.735
Exploration (12 months)	-.784
Avoidance of mother (12 months)	-.842
Factor 18 months (Eigenvalue = 23.62; Variance accounted for = 65.44%)	
Crying (18 months)	.873
Contact maintenance with mother (18 months)	.803
Resistance to stranger (18 months)	.762
Proximity seeking to mother (18 months)	.864
Exploration (18 months)	-.796
Avoidance of mother (18 months)	-.754

Note. Correlation between 12-month Factor 1 and 18-month Factor 1: r = .67*** Resistance to mother did not load on any factors.

Table 3

Associations of the 5-HTTLPR with Infant Proneness to Distress and Infant Attachment Behavior

	5-HTTLPR genotypes (mean scores with (SD))[a]					
Variable	**L/L**	**S/L**	**S/S**	**β**	**t-value**	***p***
Infant proneness to distress						
Combined Factor (12-18 months)	-.66 (.50)	.44 (1.04)	.15 (1.08)	.34	2.11	.03
Factor - 12 months	-.70 (.63)	.40 (.96)	.27 (1.09)	.32	2.01	.04
Factor - 18 months	-.63 (.59)	.45 (1.09)	-.02 (.82)	.25	1.56	.12
Infant attachment behavior						
Secure vs. insecure (12 months)	9se/4in	9se/9in	5se/3in	.05	.31	.75
Secure vs. insecure (18 months)	7se/6in	8se/10in	3se/5in	.13	.81	.42
Disorganized behavior (12 months)	3.33 (.79)	3.00 (1.24)	2.38 (1.81)	-.10	-.60	.55
Disorganized behavior (18 months)	3.81 (1.18)	3.31 (1.18)	4.08 (.97)	.08	.51	.61
Maternal risk factors						
Maternal psychosocial risk	2.08 (.76)	1.56 (.78)	1.88 (.99)	-.13	-.80	.42
Level of maternal disrupted communication	5.25 (1.22)	4.35 (1.41)	5.25 (1.50)	-.02	-.15	.88

[a]Non-imputed original values