Attachment & Human Development

ISSN: 1461-6734 (Print) 1469-2988 (Online) Journal homepage: http://www.tandfonline.com/loi/rahd20

Epigenetic modification of the oxytocin and glucocorticoid receptor genes is linked to attachment avoidance in young adults

Tsachi Ein-Dor, Willem J. M. I. Verbeke, Michal Mokry & Pascal Vrtička

To cite this article: Tsachi Ein-Dor, Willem J. M. I. Verbeke, Michal Mokry & Pascal Vrtička (2018) Epigenetic modification of the oxytocin and glucocorticoid receptor genes is linked to attachment avoidance in young adults, Attachment & Human Development, 20:4, 439-454, DOI: 10.1080/14616734.2018.1446451

To link to this article: https://doi.org/10.1080/14616734.2018.1446451

ATTACHMENT & HUMAN DEVELOPMENT, 2018
VOL. 20, NO. 4, 439–454
https://doi.org/10.1080/14616734.2018.1446451

Routledge
Taylor & Francis Group

Epigenetic modification of the oxytocin and glucocorticoid receptor genes is linked to attachment avoidance in young adults

Tsachi Ein-Dor[a], Willem J. M. I. Verbeke[b], Michal Mokry[c] and Pascal Vrtička[d]

[a]School of Psychology, Interdisciplinary Center (IDC) Herzliya, Herzliya, Israel; [b]Department of Business Economics, Erasmus School of Economics, Erasmus University Rotterdam, Rotterdam, The Netherlands; [c]Division of Pediatrics, University Medical Centre Utrecht, Utrecht, The Netherlands; [d]Department of Social Neuroscience, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany

ABSTRACT

Attachment in the context of intimate pair bonds is most frequently studied in terms of the universal strategy to draw near, or away, from significant others at moments of personal distress. However, important interindividual differences in the quality of attachment exist, usually captured through secure versus insecure – anxious and/or avoidant – attachment orientations. Since Bowlby's pioneering writings on the theory of attachment, it has been assumed that attachment orientations are influenced by both genetic and social factors – what we would today describe and measure as gene by environment interaction mediated by epigenetic DNA modification – but research in humans on this topic remains extremely limited. We for the first time examined relations between intra-individual differences in attachment and epigenetic modification of the oxytocin receptor (*OXTR*) and glucocorticoid receptor (*NR3C1)* gene promoter in 109 young adult human participants. Our results revealed that attachment avoidance was significantly and specifically associated with increased *OXTR* and *NR3C1* promoter methylation. These findings offer first tentative clues on the possible etiology of attachment avoidance in humans by showing epigenetic modification in genes related to both social stress regulation and HPA axis functioning.

ARTICLE HISTORY
Received 5 December 2017
Accepted 25 February 2018

KEYWORDS
Proximity seeking under stress; *OXTR*; *NR3C1*; epigenetics; attachment

Introduction

Attachment represents one of the most fundamental human behaviors (Insel & Young, 2001). From the moment of birth and continuing through all stages of life, its biological function is to enhance the chances of survival in times of danger and need through proximity seeking to significant others (Mikulincer & Shaver, 2007). Attachment can therefore also be understood as a universal social defense strategy (Ein-Dor & Hirschberger, 2016): humans seek support from others when faced with danger to improve their ability to deal with threat through effective cooperation and by utilizing the strength of numbers (Axelrod & Hamilton, 1981). Attachment theory, however, is not only concerned with the establishment of attachment bonds per se,

CONTACT Pascal Vrtička vrticka@cbs.mpg.de Department of Social Neuroscience, Max Planck Institute for Human Cognitive and Brain Sciences, PO BOX 500 355, 04303 Leipzig, Germany
Supplemental data can be accessed here.

but critically emphasizes interindividual variability in the quality of attachment, the latter usually being captured by the emergence of secure versus insecure – anxious and/or avoidant – attachment orientations. Secure attachment is typically characterized by the primary or secure-based strategy of social support-seeking under threat and thus the successful use of social resources to cope with stress. In contrast, attachment anxiety and avoidance are associated with the emergence of secondary attachment strategies because of inconsistency of caregivers' responses, insensitivity, and/or unavailability. These secondary attachment strategies aim at either intensifying proximity seeking to deal with mounting distress through attachment system hyperactivation in the case of attachment anxiety, or deactivating the attachment system to preclude sustained distress in the case of attachment avoidance, and are thought to be mediated by increased vigilance to, or distancing from, threat- and attachment-related cues, respectively (Mikulincer & Shaver, 2007).

When it comes to possible mechanistic explanations for the emergence of the above interindividual differences in attachment quality in terms of secure versus insecure, avoidant and/or anxious attachment during human development, there has been a remarkable change of perspective during the last decades. While initially, emphasis has been on the family and thus the environment as the primary agent of "socialization", a shift occurred at the end of the 20th century towards genetic predispositions – or put differently, "from a primarily psychosocial model of child and adult development to a genetic-biological frame of reference that often a priori excludes consideration of child-parent relationships" (Fonagy, 2001, p. 432). Only more recently, the two diverging psychosocial versus genetic-biological views have been consolidated by acknowledging that the emergence of attachment orientations during human development likely represents a prototypical gene by environment interaction, in that "the manner in which environment is experienced will act as a filter in the expression of genotype into phenotype" (Fonagy, 2001, p. 434).

In line with the above theoretical considerations, initial applied research into attachment development and the associated intergenerational transmission of attachment primarily focused on environmental factors within the family context, and in particular caregiver sensitivity. Such approach, however, could only account for a limited amount of variance in the acquired data, which lead researchers to postulate a so-called "transmission gap", the latter suggesting that a purely environmental explanation of attachment transmission and formation was insufficient (Van IJzendoorn, 1995; Verhage et al., 2016). As an alternative approach to explain additional variance, subsequent attempts aimed at disclosing predominantly genetic mechanisms underlying attachment in terms of allelic DNA variation (i.e. specific polymorphisms in candidate genes). Such approach, however, also provided only modest to no results (see e.g. Bakermans-Kranenburg & van IJzendoorn, 2016), both regarding the formation of early infant–mother attachment relationships (Leerkes et al., 2017; Roisman & Fraley, 2008), as well as the characterization of attachment styles during adulthood (Gillath, Shaver, Baek, & Chun, 2008). An experimental shift in focus on a gene by environment interaction has consequently been suggested by means of epigenetic mechanisms (Champagne, 2008), an approach inspired by the seminal work on the effect of maternal care in terms of licking and grooming as well as arched-back nursing on offspring anxiety in rats related to hypothalamus-pituitary-adrenal (HPA) axis functioning and glucocorticoid signaling (Weaver et al., 2004). Within this context, the most widely studied epigenetic process in animals as well as humans is DNA methylation, which refers to a chemical modification of DNA bases at so-called CpG islands close to or within gene promoters, higher methylation levels usually being associated

with lower rates of gene transcription and thus functioning (Allis & Jenuwein, 2016). Despite clear theoretical assumptions associating attachment formation in humans with epigenetic processes, and promising findings of intergenerational attachment transmission in animal models related to DNA methylation as a function of maternal care, research in humans regarding possible determinants of interindividual differences in attachment based on epigenetics remains extremely limited and thus constitutes a very new field of research. So far, we are only aware of four separate studies in humans showing a direct link between measures of attachment and the extent of DNA methylation (Bosmans, Young, & Hankin, 2018; Haas et al., 2016; Mulder et al., 2017; Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010). Haas et al. and Bosmans et al. used the Attachment Style Questionnaire (ASQ) or the Experiences of Close Relationships – Relationship Structures Questionnaire (ECR-RS) as self-report measures of attachment style, and report positive relations between attachment anxiety and increased DNA methylation, either regarding *OXT* encoding the human structural gene for the neuropeptide oxytocin, or the *NR3C1* gene encoding the human glucocorticoid receptor. While Haas et al. (2016) interpret their findings as attachment security being associated with lower *OXT* methylation and thus higher *OXT* expression, Bosmans et al. (2018) assume that more stressed children who experienced less maternal support report increased anxious attachment when their *NR3C1* gene is highly methylated. In turn, van IJzendoorn et al. administered the (Berkeley) Adult Attachment Interview (AAI), and found higher levels of methylation of the serotonin transporter (*5HTT*) promoter in association with increased risk of unresolved responses to loss or other trauma in carriers of the usually protective *5HTTLPR II* variant. Finally, by applying the strange situation paradigm (SSP) in 14-month-old children, Mulder et al. examined the association between children's attachment classifications based on behavior as well as cortisol reactivity during the SSP and methylation in the FK506 binding protein 51 (*FKBP5*). The authors report that *FKBP5* methylation moderates the associations of *FKBP5* genotype and resistant attachment with cortisol reactivity. Although the above studies offer first insights into possible epigenetic mechanisms associated with attachment, they only looked at one candidate gene at a time. Furthermore, the two available studies that used self-report questionnaires only tested for associations between gene methylation and attachment anxiety without considering attachment avoidance (Bosmans et al., 2018), or performed two separate regression analyses for attachment anxiety and avoidance, respectively (Haas et al., 2016). Consequently, the reported effects could have been driven by attachment insecurity more generally, as an explicit and direct differentiation between the two insecure attachment orientations is missing in both cases.

In the present investigation, we therefore aimed at bridging the gap in the literature on the epigenetic basis of attachment by using self-report questionnaires. To do so, we focused on the two genes already used in the two available studies also employing self-report measures of attachment (Bosmans et al., 2018; Haas et al., 2016) within the same ($N = 109$) healthy young adults. On the one hand, we examined methylation of the promoter region of *OXTR* encoding the human oxytocin receptor as part of the oxytocin signaling pathway. On the other hand, we assessed methylation of the promoter region of *NR3C1* encoding the human glucocorticoid receptor as part of the HPA axis involved in the stress response. Besides relying on the extant literature (see above), the selection of the two candidate genes was motivated by the assumptions that attachment primarily represents a social defense or survival system (Ein-Dor & Hirschberger, 2016), and also functions a as an emotion regulation device (Mikulincer, Shaver, & Pereg, 2003). In correspondence with the "tend and

befriend" model describing the biobehavioral bases of affiliation under stress (Taylor, 2006), oxytocin (in conjunction with dopamine and endogenous opioids) likely plays an important role in the basic drive to establish supportive and comforting (i.e. positive) relationships, as well as in the stress-reducing effect of such positive social relationships under threat (Feldman, 2017; Insel & Young, 2001; MacDonald & MacDonald, 2010). In turn, *NR3C1* encoding the human glucocorticoid receptor generally reflects HPA axis functioning and thus the bodily response to threat in terms of stress, but particularly the negative feedback loop involved in stress regulation by which the glucocorticoid receptor binds cortisol at the hypothalamus and pituitary to inhibit further release of cortisol, and thereby prevents the damaging effects of extreme or chronic HPA axis activation (Tyrka, Ridout, & Parade, 2016). Empirical data published after the postulation of the above affiliative responses to stress model (Taylor, 2006) supports the notion that positive social relationships during both tend and befriend interactions in humans are neurally encoded in reward-related brain areas and that such neural encoding is likely associated with oxytocin (Feldman, 2017; Gordon et al., 2008; Kim et al., 2017; Li, Chen, Mascaro, Haroon, & Rilling, 2017; Strathearn, Fonagy, Amico, & Montague, 2009; Vrtička, Andersson, Grandjean, Sander, & Vuilleumier, 2008; Wittfoth-Schardt et al., 2012). In addition, there is evidence that acute stress increases prosocial behavior (von Dawans, Fischbacher, Kirschbaum, Fehr, & Heinrichs, 2012). Furthermore, experimental paradigms examining the influence of positive social relationships on behavioral, physiological, and neural responses to stress found a negative relationship between the two variables. During situations of threat or threat anticipation, the availability of positive social contacts (through priming, photographs, or physical presence) not only entailed diminished threat-related responses, but the extent of beneficial influence of positive social contacts on threat-related responses was modulated by the subjectively perceived degree of relationship closeness/quality or participants' attachment style (Coan, Schaefer, & Davidson, 2006; Eisenberger et al., 2011; Krahe, Drabek, Paloyelis, & Fotopoulou, 2016; Norman, Lawrence, Iles, Benattayallah, & Karl, 2015; Tops, Koole, Ijzerman, & Buisman-Pijlman, 2014; Weisman, Zagoory-Sharon, & Feldman, 2013). Concerning a gene by environment interaction in terms of epigenetic modification of the above positive social relationship formation and social stress regulation, as well as HPA axis negative feedback loop systems, accumulating evidence generally points to a role of *OXTR* and *NR3C1* in animals as well as in humans, particularly in the context of early life adversity and stressful life experiences (Bockmuhl et al., 2015; Gouin et al., 2017; Heim & Binder, 2012; Kumsta, Hummel, Chen, & Heinrichs, 2013; Lupien, McEwen, Gunnar, & Heim, 2009; Murgatroyd, Wu, Bockmuhl, & Spengler, 2010; Puglia, Lillard, Morris, & Connelly, 2015; Tyrka et al., 2016; Ziegler et al., 2015).

Regarding the specific role of attachment in this epigenetic model of social proximity-seeking and stress regulation mediated by *OXTR* and *NR3C1*, however, no dedicated theoretical framework and only very limited empirical data in humans are available to date. According to the considerations discussed above in the context of attachment theory (Mikulincer & Shaver, 2007), social defense theory (Ein-Dor & Hirschberger, 2016), and the "tend and befriend" model describing the biobehavioral bases of affiliation under stress (Taylor, 2006), we would nonetheless postulate that attachment security should be characterized by a well-functioning social proximity-seeking system, particularly during stress, and effective stress regulation through positive social relationships. This pattern underlying attachment security should therefore be associated with low

OXTR and *NR3C1* promoter methylation and thus higher gene expression/functioning. In contrast, attachment avoidance manifests itself by compulsive self-reliance and reluctance to seek out social contact, especially under stress, entailing a lack of ability to withstand high levels of (especially social) stress or prolonged exposure to stressors (Vrtička, 2017; Vrtička & Vuilleumier, 2012). We therefore expected to find a higher degree of *OXTR* and *NR3C1* promoter methylation and thus lower functioning in association with increasing attachment avoidance scores. Finally, attachment anxiety is associated with compulsive dependence on others to (co-)regulate stress and a resulting hypervigilance to signs of threat and attachment figure unavailability. We therefore predicted a lower degree of *OXTR* (i.e. higher gene functioning) but a higher degree of *NR3C1* (i.e. lower gene functioning) promoter methylation in relation to increasing attachment anxiety scores.

Methods

Participants

One-hundred-and-nine participants (56 women, 53 men; age range from 20 to 28, $M = 23.75$, $SD = 1.56$) were recruited for the present study. Participants gave written informed consent prior to, and obtained credit points for, participation. The study was approved by the Erasmus University's IRB (2017/04/10-0548wve).

Attachment measure

Attachment anxiety and avoidance were measured with 10 self-report items on a scale from 1 = "Does not describe me at all" to 7 = "Describes me very well". The respective items were derived from the Adult Attachment Scale (AAS) based on the original self-report measure of adult romantic attachment (Hazan & Shaver, 1987) that was later on revised (Collins & Read, 1990), and can be found in Supplementary Table 1. Attachment styles were calculated from the self-report items in a dimensional manner (i.e. one value per participant for anxiety and avoidance, respectively), with attachment security being characterized by low scores on both the anxiety and avoidance dimension. In turn, a fearful avoidant attachment style emerges when scores on both the anxiety and avoidance dimensions are high. Reliability of the attachment measure was high for anxiety ($\alpha = .73$) and avoidance ($\alpha = .77$).

Gene promoter methylation

Saliva for qMethyl analysis was collected using Oragene DNA OG-500 kit (DNA Genotec Inc) and isolated using prepIT L2P (DNA Genotec Inc). The % methylation of amplicons overlapping with the CpG island and adjacent to the gene promoter of interest was assayed using OneStep qMethyl kit (Zymo Research, Irvine, CA, USA). Shortly, 10 ng of global DNA was incubated in the presence (test reaction) or absence (reference reaction) of methyl sensitive restriction enzymes (*AccII, HpyCH4IV, and HpaII)* at 37 °C for 2 h, followed by real-time reverse transcription PCR (RT-PCR) as described in the manufacturer's instructions. Percentage methylation was calculated using the formula 100 * 2^(-ΔCt), where ΔCt is the Ct value from the test reaction minus the Ct value from the reference reaction. Percentage

methylation is relative to each experiment (Rao, Keleshian, Klein, & Rapoport, 2012). For *OXTR*, the primer pairs used for qMethyl qPCR reaction were: forward 5′-CAC TCC CTG AAC TTC CAC AG-3′, and reverse 5′-GTG ACT TCT CGG GAT TGA GAC-3′. The amplicon used is located in the 1st intron, position chr3: 8,810,552–8,810,784 in GRCh37hg19 coordinates. For *NR3C1*, the primer pairs used for qMethyl qPCR reaction were: forward 5′-ACT TCG AAA GGG GCT ACG G-3′, and reverse 5′-CCA AAG TAC GTA TGC GCC G-3′. The amplicon used spanned the end of 1B and beginning of 1F site/locus, position chr5: 142,783,805–142,783,945 in GRCh37hg19 coordinates (see Figure 1). According to the manufacturer's instructions, the accuracy of the OneStep qMethyl-Lite procedures for determining *NR3C1* and *OXTR* promoter methylation percentage was validated by including the human methylated & non-methylated DNA standards with the control MGMT primers comprised in the analysis kit. Furthermore, the specificity of each primer pair was validated by melting curve analysis and agarose gel electrophoresis of amplicons after PCR reaction, resulting in single bands matching the predicted size.

Statistical analyses

Associations between attachment anxiety and avoidance as well as *OXTR* and *NR3C1* promoter methylation were calculated using hierarchical multiple regression analyses in SPSS (IBM SPSS Statistics for Windows, Version 24.0. Armonk, NY). First, anxiety (AX) and avoidance (AV) scores were entered separately, and in a subsequent step, their interaction term (AV*AX) was added. In the case of *OXTR*, we furthermore controlled for sex by adding it as an additional factor to the hierarchical multiple regression procedure. Significant interactions between *NR3C1* and/or *OXTR* promoter methylation and AV*AX

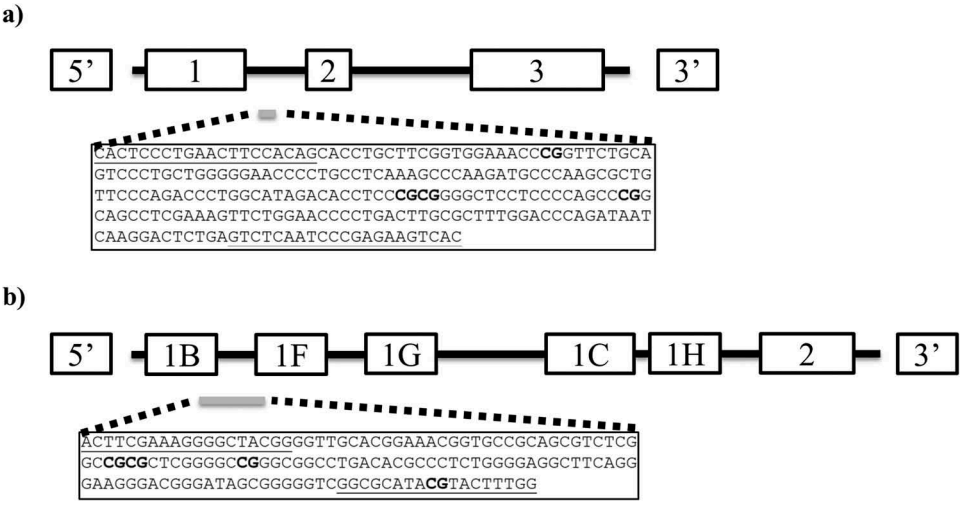

Figure 1. Illustration of (parts of) the human *OXTR* (a) and *NR3C1* (b) genes and the CpG regions analyzed by qMethyl analysis (highlighted below the gene parts in gray). The enlarged box describes the assessed amplicons, and in particular the CpG sites (4 per gene) indicated in bold. The used 5′ and 3′ primers are underlined.

were further examined using simple slopes analyses (Hayes, 2013). To avoid multicollinearity, attachment scores were centered around the sample mean.

Results

Average scores for attachment anxiety ranged from 1.40 to 6.00 (M = 2.91, SD = 1.11), and avoidance from 1.00 to 5.50 (M = 3.03, SD = 1.22). Anxiety and avoidance scores were significantly positively correlated, $r_{(107)} = .21$, $p = .025$, and there were no gender differences in anxiety, $t_{(107)} = -1.18$, $p = .24$, and avoidance, $t_{(107)} = -0.37$, $p = .72$.

The extent of *OXTR* promoter methylation ranged from 4.15 to 32.99% (M = 13.72, SD = 5.95), and *NR3C1* promoter methylation ranged from 1.07 to 12.16% (M = 3.27, SD = 1.89). *OXTR* and *NR3C1* promoter methylation were not significantly correlated, $r_{(107)} = -.07$, and there were no gender differences in promoter methylation for *OXTR*, $t_{(107)} = -1.50$, $p = .14$, or *NR3C1*, $t_{(107)} = -1.28$, $p = .20$.

The results of the two multiple regression analyses calculated to assess the relation between the degree of promoter methylation and attachment anxiety and avoidance scores are summarized in Table 1. Because for both genes, we observed a significant interaction between degree of promoter methylation and AV*AX, additional simple slopes analyses were conducted to decompose the AV*AX interaction (Hayes, 2013) and their outcomes are illustrated for better interpretability of findings in Figure 2. Due to known sex-differences, the regression analysis for *OXTR* is shown by including the factor sex. We also re-calculated the regression analyses for *NR3C1* by including the factor sex, and the findings did not significantly differ.

To shortly summarize the main findings, we observed that the degree of *OXTR* and *NR3C1* promoter methylation was highest for attachment avoidance (i.e. people scoring low on attachment anxiety and high on avoidance) as compared to all other attachment orientations (secure, anxious, and fearful avoidant). Specifically, the analyses revealed significant interactions between attachment anxiety and avoidance when predicting the degree of *OXTR* and *NR3C1* promoter methylation. Simple slopes tests indicated that only among people scoring low on attachment anxiety (one standard deviation below the mean), the higher the attachment avoidance score, the greater the degree of *OXTR* promoter methylation, $b = 2.45$, $\beta = .44$, $t = 2.06$, $p = .04$, and *NR3C1* promoter methylation, $b = 0.54$, $\beta = .40$, $t = 2.59$, $p = .01$. In contrast, the associations between attachment avoidance and the degree of *OXTR* promoter methylation, $b = -0.77$, $\beta = -.13$, $t = -0.97$, $p = .33$, and *NR3C1* promoter methylation, $b = 0.01$, $\beta = .01$, $t = 0.03$, $p = .97$, were not significant among people scoring high on attachment anxiety (one standard deviation above the mean).

Discussion

Attachment bond formation is nowadays appreciated to represent a prototypical gene by environment interaction during which "the expression of individual genotypes is intrinsically linked to the relationship with the primary caregiver" (Fonagy, 2001, p. 427) – as already suggested by Bowlby's pioneering writings on the theory of attachment (e.g. Bowlby, 1969). There is, however, an apparent lack of empirical research in humans associating the emergence of attachment orientations with epigenetic mechanisms as

Table 1. Regression coefficients for the two analyses of the relation between extent of *OXTR* and *NR3C1* promoter methylation and attachment anxiety (AX) and avoidance (AV) scores. The most relevant AV*AX interaction is highlighted in italic and bold. *$p < .05$.

NR3C1		*B*	*β*	*Pratt's Index*
Step 1	AX	−0.1	−0.08	0.1
	AV	0.22	0.17	0.9
	R^2	0.03		
Step 2	AX	−0.15	−0.11	0.06
	AV	0.28*	0.22	0.48
	AV × AX	***−0.26****	***−0.21***	***0.46***
	R^2	0.07		
OXTR		*B*	*β*	*Pratt's Index*
Step 1	AX	−0.84	−0.15	0.36
	AV	−0.04	−0.01	0.01
	Sex	−2.16	−0.19	0.63
	R^2	0.05		
Step 2	AX	−1.15	−0.21	0.27
	AV	0.41	0.07	−0.03
	Sex	−2.43*	−0.22	0.38
	AX × AV	−1.04	−0.18	0.17
	Sex × AX	0.74	0.07	−0.04
	Sex× AV	−2.26	−0.19	0.25
	R^2	0.1		
Step 3	AX	−1.51*	−0.27	0.25
	AV	0.96	0.16	−0.05
	Sex	−3.19*	−0.29	0.36
	AX × AV	***−1.59****	***−0.27***	***0.19***
	Sex × AX	1.59	0.14	−0.07
	Sex × AV	−3.52*	−0.29	0.28
	Sex × AX × AV	2.74	0.24	0.03
	R^2	0.13		

specific developmental adaptations to particular social environments (Bakermans-Kranenburg & van IJzendoorn, 2016). To bridge this gap, we investigated the association between interindividual differences in attachment anxiety and avoidance and epigenetic modification in the promoter region of two genes within the same 109 healthy adult participants: (i) a gene related to proximity seeking as a social strategy to deal with stress, namely *OXTR*, and (ii), a gene involved in stress regulation through the HPA axis, namely *NR3C1*. According to attachment theory (Mikulincer & Shaver, 2007), social defense theory (Ein-Dor & Hirschberger, 2016), and the "tend and befriend" model describing the biobehavioral bases of affiliation under stress (Taylor, 2006), we predicted attachment avoidance to be associated with higher *OXTR* and *NR3C1* promoter methylation, and attachment anxiety with lower *OXTR* but higher *NR3C1* promoter methylation. Our findings confirmed our hypothesis regarding attachment avoidance by revealing a selective positive association between *OXTR* and *NR3C1* promoter methylation and attachment avoidance. We did, however, not observe any relations between *OXTR* and *NR3C1* promoter methylation and attachment anxiety.

Attachment avoidance and OXTR and NR3C1 promoter methylation

Attachment theory (Mikulincer & Shaver, 2007) generally associates attachment avoidance with deactivating strategies aiming at keeping the attachment system in a low activation state. The latter goal is thought to be maintained through distancing from

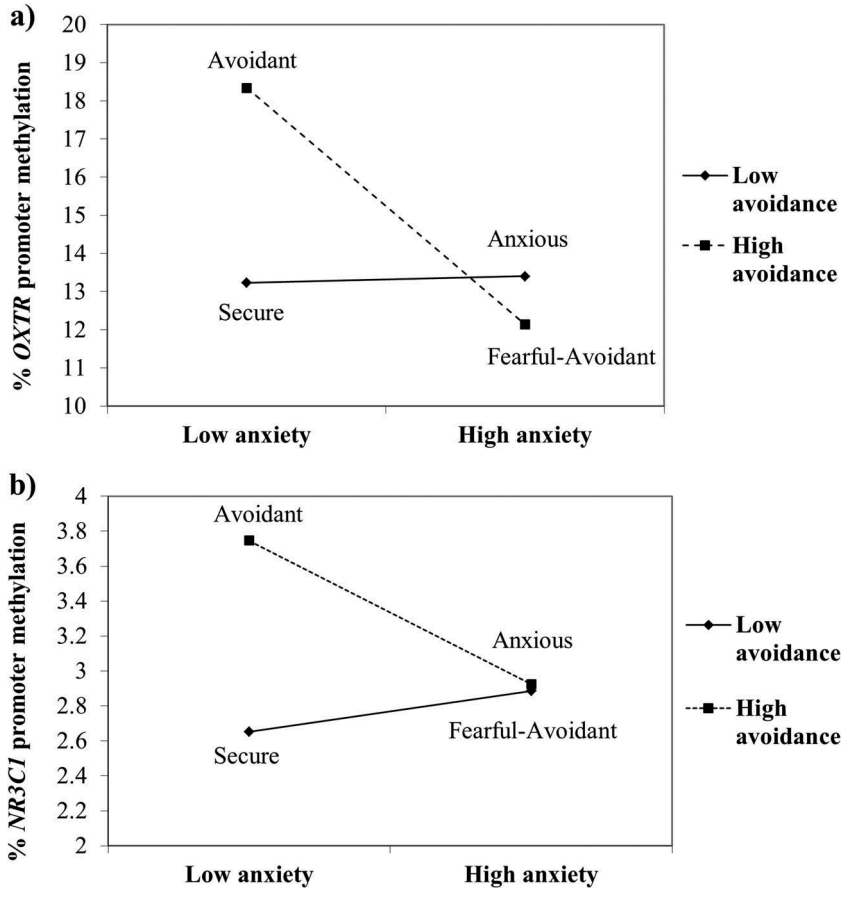

Figure 2. Illustration of the relation between the extent of *OXTR* (a) and *NR3C1* (b) promoter methylation (in %) and attachment anxiety and avoidance scores as derived from simple slopes analyses.

threat- and attachment-related cues. This view accords with the notion put forward by social defense theory (Ein-Dor & Hirschberger, 2016) that avoidantly attached individuals prefer not to rely upon others to regulate stress. A possible underlying mechanism of such reluctance to seek social proximity under stress is likely due to the fact that social interactions are not connoted with positive feelings maintained by reward-related brain circuits probably under the influence of (amongst others) oxytocin (Kim et al., 2017; Strathearn et al., 2009; Vrtička, 2017; Vrtička et al., 2008; Vrtička & Vuilleumier, 2012) so that an affiliative, prosocial response to stress (Taylor, 2006; von Dawans et al., 2012) is discouraged. Here, we for the first time provide preliminary evidence for a selective association between attachment avoidance and epigenetic modification of *OXTR* in terms of *OXTR* promoter hypermethylation: the higher the attachment avoidance score in participants scoring low on attachment anxiety (i.e. individuals known as dismissive-avoidant; Mikulincer & Shaver, 2007), the higher the *OXTR* promoter methylation. These data support the above mechanistic explanation of a lack of stress regulation through positive social contacts specifically related to attachment avoidance, and thereby

suggest a possible developmental process through a specific adaptation in terms of a gene by environment interaction.

In addition to the above positive relation between *OXTR* promoter methylation and attachment avoidance, we also observed a positive association between attachment avoidance and *NR3C1* promoter methylation: the higher the attachment avoidance score in participants scoring low on attachment anxiety, the higher the *NR3C1* promoter methylation. This pattern putatively points toward less efficient HPA axis negative feedback loop regulation (Palma-Gudiel, Cordova-Palomera, Leza, & Fananas, 2015) likely entailing a general deficiency in emotion and stress regulation (Vrtička, 2017; Vrtička & Vuilleumier, 2012). Although attachment theory (Mikulincer & Shaver, 2007) generally links attachment avoidance with secondary attachment strategies entailing the distancing from threat- (and attachment-) related cues, this does not mean that avoidantly attached individuals do not show any stress response. In fact, even during the strange situation paradigm in infants, avoidantly classified individuals are described as physically highly aroused during separation and/or the encounter with a stranger, but without overt external signs of such high internal distress (Gander & Buchheim, 2015). Our own fMRI findings in adults accord with this description, suggesting that avoidantly attached people rely upon emotion suppression rather than constructive emotion coping through, for example, cognitive re-appraisal, and that even the suppression-based emotion regulation mechanism fails particularly when negative social emotions have to be dealt with (Vrtička, Bondolfi, Sander, & Vuilleumier, 2012). Furthermore, there is evidence from structural MRI scans showing an association between attachment avoidance and bilateral hippocampal cell density reduction with the hippocampus being a crucial part of the HPA axis negative feedback loop (Quirin, Gillath, Pruessner, & Eggert, 2010). Within the context of the above extant literature, we may thus speculate that our finding of increased *NR3C1* promoter methylation as a function of attachment avoidance points toward an alteration of emotion regulation mechanisms in terms of a less efficient negative HPA axis regulation loop through a developmental epigenetic gene by environment interaction. The idea behind such process would be that, because avoidantly attached people are compulsively self-reliant and reluctant to seek out social contact especially under stress, they lack the ability to withstand high levels of stress or prolonged exposure to stressors (e.g. Berant, Mikulincer, & Florian, 2001, Ein-Dor, Mikulincer, Doron, & Shaver, 2010, Wijngaards-de Meij, Stroebe, Schut, Stroebe, van den Bout, van der Heijden, & Dijkstra, 2007).

According to the "tend and befriend" model of social affiliation under stress (Taylor, 2006), the search for positive relationships to cope with distress should result in stress reduction, which reflects the notion of attachment serving as a social defense or survival strategy (Ein-Dor & Hirschberger, 2016). Although our new epigenetic findings tentatively suggest that this process is altered through a developmental epigenetic gene by environment interaction in avoidantly attached individuals, our data cannot yet provide a comprehensive neurobiological and -physiological account as we only assessed participants' epigenetic status during adulthood and did not acquire any biological and/or physiological measures. Furthermore, in our participant sample, the degree of *OXTR* and *NR3C1* promoter methylation was not significantly related, which precludes the establishment of a direct link between social stress and negative feedback HPA axis regulation. Nonetheless, our new data offer first tentative

clues on a possible epigenetic basis of an avoidant attachment orientation that can be followed up in future experiments. Importantly, the latter experiments should replicate and extend the here reported findings by also examining physiological stress reactions and the social (versus nonsocial) regulation of stress as a function of attachment orientations (Ditzen et al., 2008; Monaco et al., 2017; Pierrehumbert, Torrisi, Ansermet, Borghini, & Halfon, 2012; Smyth et al., 2015; Ziegler et al., 2015), and ideally comprise longitudinal data acquisition so that a causal pre versus post comparison during development in terms of an epigenetic mechanism can be established.

Attachment anxiety and OXTR and NR3C1 promoter methylation

In contrast to the associations between *OXTR* and *NR3C1* promoter methylation and attachment avoidance reported above, we did not observe any specific relations between the two epigenetic markers and attachment anxiety. This stands in contrast to the two so far available investigations reporting associations between *OXT* and *NR3C1* methylation and attachment anxiety in humans (Bosmans et al., 2018; Haas et al., 2016). Furthermore, different research in human participants showed that attachment anxiety was linked to deceased hippocampal cell density (Quirin et al., 2010) as an index for impaired stress regulation – similarly to attachment avoidance (see above). One general possible interpretation of such discrepancy may be the fact that we included attachment anxiety and avoidance within the same multiple hierarchical regression model and explicitly looked for an interaction between the two attachment orientations, because genuine anxiety relates to high levels of anxiety that co-occur with low levels of avoidance, whereas genuine avoidance relates to high levels of avoidance that co-occur with low levels of anxiety. This approach was not used in the other so far available analyses – in the latter, attachment anxiety was assessed independently of avoidance (Haas et al., 2016) or attachment avoidance was not considered at all (Bosmans et al., 2018). The effects of attachment anxiety reported previously may thus have been confounded by an influence of attachment avoidance and represent a more general effect of attachment insecurity. It should also be noted here that we assessed promoter methylation of the *OXTR* gene and not the *OXT* gene as done before (Haas et al., 2016). In addition, it may also be that the examined participant samples of this and previous studies differed in their *OXTR* and/or *NR3C1* genotype (i.e. allelic difference) that has been shown to interact with DNA methylation patterns (Bell et al., 2015). Future studies should therefore ideally include more than the two candidate genes assessed here, more comprehensively covering the oxytocin signaling and HPA axis pathways, and obtain information on the participants' corresponding genotype.

General considerations and limitations

In the present study, we used a self-report measure of attachment as prominently employed in social psychology research. In the field of developmental psychology, however, the use of narrative-based measures of adult attachment representations is more common – for example the Adult Attachment Interview (AAI) or the Attachment

Script Assessment. Research shows that "social and developmental psychological measures of attachment security predict somewhat distinct – though theoretically anticipated – aspects of functioning in adult relationships" (Roisman et al., 2007, p. 678). More research is therefore needed to evaluate whether *NR3C1* and/or *OXTR* promoter methylation is also associated with the narrative-based measures of adult attachment representations.

It should also be noted here that in the present study, we only focused on two candidate genes: *NR3C1* and *OXTR*. These two candidate genes were chosen based on the theoretical considerations in association with the Social Defense Theory (SDT) and the "tend and befriend" model describing the biobehavioral basis of affiliation under stress, as well as because the HPA axis and the oxytocin system were already targeted in two other extant investigations employing self-report measures of attachment (Bosmans et al., 2018; Haas et al., 2016). There are, however, two other studies available in the literature that report associations between (i) Adult Attachment Interview (AAI) classifications and methylation in the serotonin transporter gene (*5HTT*) in adults (Van IJzendoorn et al., 2010), and (ii) 14-month-old children's attachment classifications based on behavior during the Strange Situation Procedure (SSP) and methylation in the FK506 binding protein 51 (*FKBP5*) (Mulder et al., 2017). It therefore appears that attachment orientations may influence methylation patterns of a range of genes. What is crucially lacking so far, however, is a unified theory regarding what specific epigenetic modifications in terms of methylation might be found in relation to attachment and why. Follow-up studies should thus extend the scope by including additional target genes, ideally in the same participants, and try to work out an overarching theoretical account of methylation patterns in association with attachment.

Furthermore, the reader should be aware of the fact that the assessment of epigenetic modification by means of gene (promoter) methylation generally shows considerable variation in methodology and lacks a consensus regarding selection of CpG sites – as pointed out in a recent critical review regarding the *NR3C1* gene (Palma-Gudiel et al., 2015). The above also applies to the present study as compared to the two extant investigations regarding the association between attachment and *NR3C1* and *OXT* methylation (Bosmans et al., 2018; Haas et al., 2016) that differed in *NR3C1* CpG sites and methylation detection methodology, which may – amongst other factors – account for the variability of the results of these three studies. For the future, a stronger overlap between the selection of CpG sites as well as methodology to derive DNA methylation would increase generality of the findings and thus ease cross-study comparison.

Finally, as for any correlational study, we cannot conclude from our data what is cause and what is effect regarding the association between attachment avoidance and *OXTR* and *NR3C1* promoter methylation. More research is therefore clearly needed to determine *OXTR* and *NR3C1* promoter methylation levels as a function of attachment at different developmental stages using longitudinal experimental designs.

Despite the above limitations, we think that the assessment of gene (promoter) methylation offers a promising new avenue to study gene by environment interactions in the context of attachment – particularly as compared to previous studies that primarily relied on genetic mechanisms as measured by specific polymorphisms in candidate genes with modest to no results (Gillath et al., 2008; Leerkes et al., 2017; Roisman & Fraley, 2008).

Conclusion

We for the first time report an association between *OXTR* and *NR3C1* promoter methylation and attachment avoidance in a sample of 109 young healthy adults. These findings provide tentative clues on a possible gene by environment interaction through epigenetic modification of two genes importantly involved in social responses to stress, thereby critically extending the so far extremely limited literature on the epigenetic basis of attachment. Future research is needed to replicate and extend such results to obtain a more differentiated and comprehensive understanding of the determinants of interindividual differences in attachment quality.

Acknowledgments

The research team is grateful for funding received from Erasmus University Rotterdam and the Max Planck Society in association with this research project. We would also like to thank all participants for their contribution.

Disclosure statement

No potential conflict of interest was reported by the authors.

Funding

This work was supported by the Erasmus Universiteit Rotterdam; Max-Planck-Gesellschaft.

ORCID

Pascal Vrtička http://orcid.org/0000-0002-8920-5509

References

Allis, C. D., & Jenuwein, T. (2016). The molecular hallmarks of epigenetic control. *Nature Reviews Genetics*, *17*(8), 487–500.

Axelrod, R., & Hamilton, W. D. (1981). The evolution of cooperation. *Science*, *211*(4489), 1390–1396.

Bakermans-Kranenburg, M. J., & van IJzendoorn, M. H. (2016). Attachment, parenting, and genetics. In J. Cassidy & P. R. Shaver (Eds.), *Hanbook of attachment: Theory, research, and clinical applications* (3rd ed., pp. 155–180). New York: Guilford Press.

Bell, A. F., Carter, C. S., Steer, C. D., Golding, J., Davis, J. M., Steffen, A. D., … Connelly, J. J. (2015). Interaction between oxytocin and receptor DNA methylation and genotype is associated with risk of postpartum depression in women without depression in pregnancy. *Frontiers in Genetics*, *6*. doi:10.3389/fgene.2015.00243

Berant, E., Mikulincer, M., & Florian, V. (2001). The Association Of Mothers' Attachment Style And Their Psychological Reactions To The Diagnosis Of Infant's Congenital Heart Disease. *Journal of Social and Clinical Psychology*, *20*(2), 208–232. doi:10.1521/jscp.20.2.208.22264

Bockmuhl, Y., Patchev, A. V., Madejska, A., Hoffmann, A., Sousa, J. C., Sousa, N., … Spengler, D. (2015). Methylation at the CpG island shore region upregulates NR3C1 promoter activity after early-life stress. *Epigenetics*, *10*(3), 247–257.

Bosmans, G., Young, G. F., & Hankin, B. L. (2018). NR3C1 methylation as a moderator of the effects of maternal support and stress on insecure attachment development. *Developmental Psychology, 54*(1), 29–38. doi: 10.1037/dev0000422

Bowlby, J. (1969). *Attachment and loss, Vol. 1: Attachment*. New York: Basic Books.

Champagne, F. A. (2008). Epigenetic mechanisms and the transgenerational effects of maternal care. *Frontiers in Neuroendocrinology*, *29*(3), 386–397.

Coan, J. A., Schaefer, H. S., & Davidson, R. J. (2006). Lending a hand: Social regulation of the neural response to threat. *Psychological Science*, *17*(12), 1032–1039.

Collins, N. L., & Read, S. J. (1990). Adult attachment, working models, and relationship quality in dating couples. *Journal of Personality and Social Psychology*, *58*(4), 644–663.

Ditzen, B., Schmidt, S., Strauss, B., Nater, U. M., Ehlerta, U., & Heinrichs, M. (2008). Adult attachment and social support interact to reduce psychological but not cortisol responses to stress. *Journal of Psychosomatic Research*, *64*(5), 479–486.

Ein-Dor, T., & Hirschberger, G. (2016). Rethinking attachment theory: From a theory of relationships to a theory of individual and group survival. *Current Directions in Psychological Science*, *25*(4), 223–227.

Ein-Dor, T., Mikulincer, M., Doron, G., & Shaver, P. R. (2010). The attachment paradox: how can so many of us (the insecure ones) have no adaptive advantages?. *Perspectives on Psychological Science*, *5*(2), 123–141. doi: 10.1177/1745691610362349

Eisenberger, N. I., Master, S. L., Inagaki, T. K., Taylor, S. E., Shirinyan, D., Lieberman, M. D., & Naliboff, B. D. (2011). Attachment figures activate a safety signal-related neural region and reduce pain experience. *Proceedings of the National Academy of Sciences of the United States of America*, *108* (28), 11721–11726.

Feldman, R. (2017). The neurobiology of human attachments. *Trends in Cognitive Sciences*, *21*(2), 80–99.

Fonagy, P. (2001). The human genome and the representational world: The role of early mother-infant interaction in creating an interpersonal interpretive mechanism. *Bulletin of the Menninger Clinic*, *65*(3), 427–448.

Gander, M., & Buchheim, A. (2015). Attachment classification, psychophysiology and frontal EEG asymmetry across the lifespan: A review. *Frontiers in Human Neuroscience*, *9*. doi:10.3389/fnhum.2015.00079

Gillath, O., Shaver, P. R., Baek, J. M., & Chun, D. S. (2008). Genetic correlates of adult attachment style. *Personality and Social Psychology Bulletin*, *34*(10), 1396–1405.

Gordon, I., Zagoory-Sharon, O., Schneiderman, I., Leckman, J. F., Weller, A., & Feldman, R. (2008). Oxytocin and cortisol in romantically unattached young adults: Associations with bonding and psychological distress. *Psychophysiology*, *45*(3), 349–352.

Gouin, J. P., Zhou, Q. Q., Booij, L., Boivin, M., Cote, S. M., Hebert, M., … Vitaro, F. (2017). Associations among oxytocin receptor gene (OXTR) DNA methylation in adulthood, exposure to early life adversity, and childhood trajectories of anxiousness. *Scientific Reports*, *7*. doi:10.1038/s41598-017-07950-x

Haas, B. W., Filkowski, M. M., Cochran, R. N., Denison, L., Ishak, A., Nishitani, S., & Smith, A. K. (2016). Epigenetic modification of OXT and human sociability. *Proceedings of the National Academy of Sciences of the United States of America*, *113*(27), E3816–E3823.

Hayes, A. F. (2013). *Introduction to mediation, moderation, and conditional process analysis: A regression-based approach* (Vol. 1). New York, NY: Guilford Press.

Hazan, C., & Shaver, P. (1987). Romantic love conceptualized as an attachment process. *Journal of Personality and Social Psychology*, *52*(3), 511–524.

Heim, C., & Binder, E. B. (2012). Current research trends in early life stress and depression: Review of human studies on sensitive periods, gene-environment interactions, and epigenetics. *Experimental Neurology*, *233*(1), 102–111.

Insel, T. R., & Young, L. J. (2001). The neurobiology of attachment. *Nature Reviews Neuroscience*, *2* (2), 129–136.

Kim, S., Iyengar, U., Mayes, L. C., Potenza, M. N., Rutherford, H. J. V., & Strathearn, L. (2017). Mothers with substance addictions show reduced reward responses when viewing their own infant's face. *Human Brain Mapping*, *38*(11), 5421–5439.

Krahe, C., Drabek, M. M., Paloyelis, Y., & Fotopoulou, A. (2016). Affective touch and attachment style modulate pain: A laser-evoked potentials study. *Philosophical Transactions of the Royal Society B-Biological Sciences*, *371*(1708). doi:10.1098/rstb.2016.0009

Kumsta, R., Hummel, E., Chen, F. S., & Heinrichs, M. (2013). Epigenetic regulation of the oxytocin receptor gene: Implications for behavioral neuroscience. *Frontiers in Neuroscience*, *7*. doi:10.3389/fnins.2013.00083

Leerkes, E. M., Gedaly, L. R., Zhou, N., Calkins, S., Henrichm, V. C., & Smolen, A. (2017). Further evidence of the limited role of candidate genes in relation to infant–Mother attachment outcomes. *Attachment & Human Development*, *19*(1), 76–105.

Li, T., Chen, X., Mascaro, J., Haroon, E., & Rilling, J. K. (2017). Intranasal oxytocin, but not vasopressin, augments neural responses to toddlers in human fathers. *Hormones and Behavior*, *93*, 193–202.

Lupien, S. J., McEwen, B. S., Gunnar, M. R., & Heim, C. (2009). Effects of stress throughout the lifespan on the brain, behaviour and cognition. *Nature Reviews Neuroscience*, *10*(6), 434–445.

MacDonald, K., & MacDonald, T. M. (2010). The peptide that binds: A systematic review of oxytocin and its prosocial effects in humans. *Harvard Review of Psychiatry*, *18*(1), 1–21.

Mikulincer, M., & Shaver, P. R. (2007). *Attachment in adulthood: Structure, dynamics, and change*. New York: The Guilford Press.

Mikulincer, M., Shaver, P. R., & Pereg, D. (2003). Attachment theory and affect regulation: The dynamics, development, and cognitive consequences of attachment-related strategies. *Motivation and Emotion*, *27*(2), 77–102.

Monaco, F., Monteleone, A. M., Pellegrino, F., Ruzzi, V., Fico, G., Volpe, U., & Monteleone, P. (2017). Attachment and hypothalamus-pituitary-adrenal axis functioning in patients with eating disorders. *European Psychiatry*, *41*, S551–S552.

Mulder, R. H., Rijlaarsdam, J., Luijk, M., Verhulst, F. C., Felix, J. F., Tiemeier, H., … Van IJzendoorn, M. H. (2017). Methylation matters: FK506 binding protein 51 (FKBP5) methylation moderates the associations of FKBP5 genotype and resistant attachment with stress regulation. *Development and Psychopathology*, *29*(2), 491–503.

Murgatroyd, C., Wu, Y. H., Bockmuhl, Y., & Spengler, D. (2010). Genes learn from stress How infantile trauma programs us for depression. *Epigenetics*, *5*(3). doi:10.4161/epi.5.3.11375

Norman, L., Lawrence, N., Iles, A., Benattayallah, A., & Karl, A. (2015). Attachment-security priming attenuates amygdala activation to social and linguistic threat. *Social Cognitive and Affective Neuroscience*, *10*(6), 832–839.

Palma-Gudiel, H., Cordova-Palomera, A., Leza, J. C., & Fananas, L. (2015). Glucocorticoid receptor gene (NR3C1) methylation processes as mediators of early adversity in stress-related disorders causality: A critical review. *Neuroscience and Biobehavioral Reviews*, *55*, 520–535.

Pierrehumbert, B., Torrisi, R., Ansermet, F., Borghini, A., & Halfon, O. (2012). Adult attachment representations predict cortisol and oxytocin responses to stress. *Attachment & Human Development*, *14*(5), 453–476.

Puglia, M. H., Lillard, T. S., Morris, J. P., & Connelly, J. J. (2015). Epigenetic modification of the oxytocin receptor gene influences the perception of anger and fear in the human brain. *Proceedings of the National Academy of Sciences of the United States of America*, *112*(11), 3308–3313.

Quirin, M., Gillath, O., Pruessner, J. C., & Eggert, L. D. (2010). Adult attachment insecurity and hippocampal cell density. *Social Cognitive and Affective Neuroscience*, *5*(1), 39–47.

Rao, J. S., Keleshian, V. L., Klein, S., & Rapoport, S. I. (2012). Epigenetic modifications in frontal cortex from Alzheimer's disease and bipolar disorder patients. *Translational Psychiatry*, *2*. doi:10.1038/tp.2012.55

Roisman, G. I., & Fraley, R. C. (2008). A behavior-genetic study of parenting quality, infant attachment security, and their covariation in a nationally representative sample. *Developmental Psychology*, *44*(3), 831–839.

Roisman, G. I., Holland, A., Fortuna, K., Fraley, R. C., Clausell, E., & Clarke, A. (2007). The adult attachment interview and self-reports of attachment style: An empirical rapprochement. *Journal of Personality and Social Psychology*, *92*(4), 678–697.

Smyth, N., Thorn, L., Oskis, A., Hucklebridge, F., Evans, P., & Clow, A. (2015). Anxious attachment style predicts an enhanced cortisol response to group psychosocial stress. *Stress-The International Journal on the Biology of Stress*, *18*(2), 143–148.

Strathearn, L., Fonagy, P., Amico, J., & Montague, P. R. (2009). Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology*, *34*(13), 2655–2666.

Taylor, S. E. (2006). Tend and befriend: Biobehavioral bases of affiliation under stress. *Current Directions in Psychological Science*, *15*(6), 273–277.

Tops, M., Koole, S. L., Ijzerman, H., & Buisman-Pijlman, F. T. A. (2014). Why social attachment and oxytocin protect against addiction and stress: Insights from the dynamics between ventral and dorsal corticostriatal systems. *Pharmacology Biochemistry and Behavior*, *119*, 39–48.

Tyrka, A. R., Ridout, K. K., & Parade, S. H. (2016). Childhood adversity and epigenetic regulation of glucocorticoid signaling genes: Associations in children and adults. *Development and Psychopathology*, *28*(4), 1319–1331.

Van IJzendoorn, M. H. (1995). Adult attachment representations, parental responsiveness, and infant attachment - a metaanalysis on the predictive-validity of the adult attachment interview. *Psychological Bulletin*, *117*(3), 387–403.

Van IJzendoorn, M. H., Caspers, K., Bakermans-Kranenburg, M. J., Beach, S. R. H., & Philibert, R. (2010). Methylation matters: Interaction between methylation density and serotonin transporter genotype predicts unresolved loss or trauma. *Biological Psychiatry*, *68*(5), 405–407.

Verhage, M. L., Schuengel, C., Madigan, S., Fearon, R. M. P., Oosterman, M., Cassibba, R., … van IJzendoorn, M. H. (2016). Narrowing the transmission gap: A synthesis of three decades of research on intergenerational transmission of attachment. *Psychological Bulletin*, *142*(4), 337–366.

von Dawans, B., Fischbacher, U., Kirschbaum, C., Fehr, E., & Heinrichs, M. (2012). The social dimension of stress reactivity: Acute stress increases prosocial behavior in humans. *Psychological Science*, *23*(6), 651–660.

Vrtička, P. (2017). The social neuroscience of attachment. In A. Ibáñez, L. Sedeño, & A. M. García (Eds.), *Neuroscience and social science*. Cham: Springer International Publishing.

Vrtička, P., Andersson, F., Grandjean, D., Sander, D., & Vuilleumier, P. (2008). Individual attachment style modulates human amygdala and striatum activation during social appraisal. *Plos One*, *3*(8). doi:10.1371/journal.pone.0002868

Vrtička, P., Bondolfi, G., Sander, D., & Vuilleumier, P. (2012). The neural substrates of social emotion perception and regulation are modulated by adult attachment style. *Social Neuroscience*, *7*(5), 473–493.

Vrtička, P., & Vuilleumier, P. (2012). Neuroscience of human social interactions and adult attachment style. *Frontiers in Human Neuroscience*, *6*. doi:10.3389/fnhum.2012.00212

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J., … Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience*, *7*(8), 847–854.

Weisman, O., Zagoory-Sharon, O., & Feldman, R. (2013). Oxytocin administration alters HPA reactivity in the context of parent-infant interaction. *European Neuropsychopharmacology*, *23*(12), 1724–1731.

Wijngaards-de Meij, L., Stroebe, M., Schut, H., Stroebe, W., van den Bout, J., van der Heijden, P . G. M., & Dijkstra, I. (2007). Patterns of attachment and parents' adjustment to the death of their child. *Personality And Social Psychology Bulletin*, *33*(4), 537–548. doi:10.1177/0146167206297400

Wittfoth-Schardt, D., Grunding, J., Wittfoth, M., Lanfermann, H., Heinrichs, M., Domes, G., … Waller, C. (2012). Oxytocin modulates neural reactivity to children's faces as a function of social salience. *Neuropsychopharmacology*, *37*(8), 1799–1807.

Ziegler, C., Dannlowski, U., Brauer, D., Stevens, S., Laeger, I., Wittmann, H., … Domschke, K. (2015). Oxytocin receptor gene methylation: Converging multilevel evidence for a role in social anxiety. *Neuropsychopharmacology*, *40*(6), 1528–1538.