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Separation experiences in childhood and methylation of the oxytocin gene (OXT) and vasopressin gene (AVP) in monozygotic twins

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Abstract

Separation experiences in childhood are a form of early-life stress (ELS) and have a life-long impact on an individual (Kraaijenvanger et al., 2020). Previous research suggests that DNA methylation mediates the relationship of ELS and adverse outcomes in later life (Weaver et al., 2004). ELS has been empirically linked to alteration of the oxytocinergic and vasopressin-nergic systems which influence social behavior and stress-responsivity (Kompier et al., 2019). These systems, in turn, are partly controlled by the expression of the oxytocin gene (OXT) and the vasopressin gene (AVP) which can be altered by methylation (Gimpl & Fahrenholz, 2001). Thus, our objective was to investigate the effect of separation experiences in childhood on the DNA methylation of OXT and AVP, and how the family environment and the age at separation influence it. Data of 149 monozygotic twins from the Swedish Adoption/Twin Study of Aging sample were analyzed. Fifty-eight twins were reared apart and 91were reared together. Before correcting for multiple-testing we observed significant effects of rearing status, family conflict and age at the time of separation on the methylation of single CpG sites of OXT and AVP. These results are in line with previous research and may point to a resiliency mechanism of OXT and AVP methylation after ELS which may be age-related in OXT. Nevertheless, findings need to be interpreted with caution due to lacking significance after correcting for multiple testing.

Keywords: Epigenetics, early-life stress, childhood separation, oxytocin gene (OXT), vasopressin gene (AVP), methylation, family environment, conflict

Separation experiences in childhood and methylation of the oxytocin (OXT) and vasopressin (AVP) genes in monozygotic twins

Over the last decades, a plurality of studies on both humans and animals has demonstrated that early-life stress (ELS) can have long-lasting, negative effects on the organism (Danese & McEwen, 2012; Ehlert, 2013; Kaufman et al., 2000; Kraaijenvanger et al., 2020; Pechtel & Pizzagalli, 2011). Among the many observed changes after ELS are: increased responsivity to stress (Jones-Mason et al., 2019; Kaufman et al., 2000), poorer cognitive abilities (Goodman et al., 2019; Loman et al., 2009), altered reward processing (Herzberg & Gunnar, 2020), impaired emotion perception and regulation (Herzberg & Gunnar, 2020), reduced relationship quality (Flynn et al., 2014), sleeping problems (Baiden et al., 2015) and poorer mental (Green et al., 2010) and physical health (Danese & McEwen, 2012; Ehlert, 2013).

ELS can be caused by many different events. One more severe type of ELS is childhood separation. Many children worldwide experience temporary or permanent separation from an attachment figure, be it on the flight from war or prosecution (UNICEF, 2019), on country borders (Jones-Mason et al., 2019) or through the death or detention of a parent (Haine et al., 2008). Being separated from an attachment figure is a very stressful event for a child, especially at a young age (Jones-Mason et al., 2019). Already short separations elicit a strong stress-response in an infant (Ahnert et al., 2004). Long-term separation usually has an enduring impact on a child's life and mental health (Jones-Mason et al., 2019). Besides the aforementioned consequences of ELS, childhood separation is specifically associated with insecure attachment and attachment anxiety (Bowlby, 1978; Foster et al., 2003; Jones-Mason et al., 2019). Attachment experiences in childhood, including separation, shape future social relationships (Jones-Mason et al., 2019). The disruption of attachment caused by separation can even exert its

effect on the next generation since in many cases the offspring of parents with an insecure attachment style also develops insecure attachment patterns (Verhage et al., 2016).

More recent research showed that part of the relation between ELS and its adverse outcomes could be traced back to epigenetic processes throughout the genome (Weaver et al., 2004). One of the best-studied epigenetic modifications is DNA methylation. It occurs when a methyl group is added to a so called CpG site – a cytosine base, which is followed by a guanine base in the DNA sequence (Bird, 1986; Moore et al., 2013). This methylation can alter the expression of the gene which can explain interpersonal differences in the phenotype (Moore et al., 2013; Razin & Cedar, 1991). Previous epigenetic research in relation to ELS mainly focused on genes that are connected to the serotonergic system (Jiang et al., 2019; Vinkers et al., 2015) and hypothalamic–pituitary–adrenal axis (HPA axis; Jiang et al., 2019; Vinkers et al., 2015) since those systems are altered by ELS and involved in the emergence of psychopathology (Vinkers et al., 2015). However, there are also many other genes, which are promising candidates for epigenetic research on ELS. The consequences of childhood separation might be mediated by the methylation of genes that are relevant for social behavior as well as stress responsivity. Possible candidates for this are the oxytocin gene (OXT) and the arginine vasopressin gene (AVP). These genes control the functioning of the neuropeptides oxytocin and vasopressin within the body (Gimpl & Fahrenholz, 2001).

Oxytocin and vasopressin are synthesized in the hypothalamus and released from the posterior pituitary (Meyer-Lindenberg et al., 2011). Both are structurally related and can bind to each other's receptors (Carter, 2017). Since oxytocin-like and vasopressin-like peptides can be found in all vertebrates, with similarly constructed systems, both neuropeptides are thought to have evolutionary significance (Gimpl & Fahrenholz, 2001). They are most commonly known

for being involved in complex behaviors of mammals such as sexual behavior, bonding, and parenting (Carter, 2017; Ebstein et al., 2009). Besides social behavior they are also associated with cognitive performance, cardiovascular control, and stress responsivity (Gimpl & Fahrenholz, 2001; Meyer-Lindenberg et al., 2011). They play a vital role in the human stress system where they function as antagonists: While oxytocin was found to have an anxiolytic effect by soothing the activity of the HPA-axis, vasopressin enhances its activity and is thereby anxiogenic (Heinrichs et al., 2009; Neumann & Landgraf, 2012; Stoop, 2012).

Separation experiences in early childhood were found to reduce the anxiolytic effect of oxytocin (Meinlschmidt & Heim, 2007). Furthermore, studies on rodents demonstrated that ELS alters the oxytocinergic and vasopressinergic systems (Kompier et al., 2019). Imbalances within both the oxytocin and the vasopressinergic system are linked to psychopathology in humans (Cochran et al., 2013; Gimpl & Fahrenholz, 2001; Marazziti & Dell'Osso, 2008; Neumann & Landgraf, 2012). It is yet not fully understood how ELS leads to mentioned changes in the oxytocinergic and vasopressinergic systems (Kompier et al., 2019). As outlined prior, DNA methylation may be an important mediator. Altered expression of the genes controlling the oxytocinergic and vasopressinergic systems is suspected to impact the oxytocinergic and vasopressinergic systems and thereby social and emotional functioning and possibly mental well-being (Neumann & Landgraf, 2012; Young, 2001). The key role of the oxytocin and vasopressin receptor genes has already been identified in many studies (Meyer-Lindenberg et al., 2011). The OXT and AVP, however, received less empirical attention.

One study found that higher methylation of OXT was associated with less secure attachment, a reduced ability to recognize emotional facial expression, reduced activity in brain areas essential for social-cognitive functioning (superior temporal sulcus, fusiform gyrus, and

inferior frontal gyrus), and reduced gray matter volume within the right fusiform gyrus (Haas et al., 2016). Another study observed OXT hypomethylation in relation to stressful life events (Sanwald et al., 2020). Hecker et al. (2016) reported a significant effect of ELS in the form of childhood abuse on the methylation of one AVP CpG site (cg03279206). Concerning the intergenic region (IGR) between AVP and OXT, rodent studies showed that the IGR downstream of the AVP gene corresponds to both the AVP and the OXT gene expression (Fields et al., 2003; Gainer et al., 2001). A relationship between ELS and hypomethylation of the IGR was identified (Murgatroyd et al., 2009; Murgatroyd & Spengler, 2014). The only known study on humans in the context of both OXT and AVP methylation found reduced methylation of the IGR in depressed mothers (King et al., 2017).

The present study will contribute to the understanding of the relationship between ELS and DNA methylation by investigating the effect of separation experiences on the methylation of OXT and AVP. We will use a unique sample of monozygotic twins with some reared apart and some reared together in childhood. We hypothesize that reared apart twins show different patterns of methylation of OXT, AVP and IGR than reared together twins. It is also expected that within the group of reared apart twins, the methylation of OXT, AVP and IGR is influenced by the family environment and the age at the time of the separation; the younger the more changes in methylation.

Method

Participants

Data were used from the Swedish Adoption/Twin Study of Aging sample (SATSA), a longitudinal study conducted between 1984 and 2010 to investigate the genetic and environmental factors underlying individual differences in aging (Pedersen et al., 1991). The data included 163 monozygotic twins. We only looked at the data of monozygotic twins to control for genetic variance. Due to missing separation data 14 individuals had to be excluded. Thus, our sample size consisted of 149 individuals (reared apart= 58; reared together= 91). The mean age at the time of the blood sample collection was 67.824 years (SD= 8.997; age range= 51.200 – 91.822). All participants gave their informed consent to participate.

Assessment and Measures

DNA Methylation. Blood samples of all participants were collected, and DNA was extracted. 200 ng of DNA were bisulfite converted via the EZ-96 DNA MagPrep methylation kit (Zymo Research Corp., Orange, CA, USA), followed by a genome-wide analysis of DNA methylation via the Human Methylation 450K array (Illumina Inc., San Diego, CA, USA). Thereby data of 390,894 autosomal CpG-sites were retrieved. Then the R package RnBeads was used to process raw methylation data (Wang et al., 2018). Using the genome browser from UCSC with the GRCh37/hg19 built, OXT gene was defined as: chromosome 20, base pairs 3,071,620-3,072,517 and AVP gene was defined as: chromosome 20, base pairs 3,082,556-3,084,724 (2020). 12 CpG sites on OXT were identified, 12 CpG sites on AVP and three in the IGR. (A full list of the CpG sites analyzed in this study can be found in Appendix A.) High methylation values indicate high DNA methylation and vice versa.

Rearing status. Twins were asked how old they were at the time of their separation. Those twins that were separated before the age of eleven were coded as reared apart while those separated after the age of eleven were coded as reared together. The age of eleven was used to be able to look at separation effects before puberty.

Age at the time of separation. Data on the age at the time of separation were collected by simply asking the respondents how old they were when they were separated.

Family Environment. The family environment was assessed by the Family Environment Scale (Moos & Moos, 2009), a 90-item inventory measuring three dimensions of family characteristics (Relationship, Personal Growth, and System Maintenance). We used two subscales of the Relationship Dimension that were of particular interest in the light of our research question: cohesion (degree of commitment and support family members provide for one another; Moos & Moos, 2009) and conflict (amount of openly expressed anger and conflict among family members; Moos & Moos, 2009). The conflict subscale has good internal consistency (Cronbach's alpha = .72; Boyd et al., 1997). The cohesion subscale is not far behind with a Cronbach's alpha at .67 (Boyd et al., 1997). Both subscales consisted of five items each. Cohesion and conflict values for each individual were determined by the sum of their items. The maximum value for both cohesion and conflict is 25. High values on the cohesion scale indicate high family cohesion and vice versa. High values on the conflict scale indicate low family conflict and vice versa.

Control variables. Since previous research reported an effect of gender (Cortes et al., 2019; Kompier et al., 2019), alcohol use (Zahs et al., 2012), smoking (Corley et al., 2019), and age at the time of blood collection (Jansen et al., 2019) on DNA methylation, we included them as control variables. Data on both gender (female vs. male) and the alcohol use (yes vs. no) were

dichotomous, while three categories were differentiated for smoking (never smoked vs. former smoker vs. current smoker), and age at the time of blood collection was a continuous variable.

Data analysis

For each gene (OXT and AVP) and the IGR a separate multivariate analyses of covariance (MANCOVA) was performed with the rearing status as between group factor and the identified CpG sites as dependent variables. We controlled for gender, smoking behavior, alcohol use, age at the time of blood collection, and family environment by adding these variables as covariates. To gain additional insight into the effect of separation experiences as an environmental factor on DNA methylation we conducted the same analysis on twin pair level. For this, we introduced *delta variables*. This means that for all variables in our analyses, on which the twins of a twin pair would differ from one another, we determined the differences between twin A and twin B. Delta variables for all continuous variables (methylation, age at the time of separation, age at the time of blood collection, cohesion, conflict) were created by calculating the absolute difference between twin A and twin B of each twin pair (e.g. abs(methylation of cg04731988 of Twin A minus methylation of cg04731988 of Twin B)). For each categorial variable (smoking behavior, alcohol use) we used dichotomous coding to ascertain the difference between twin A and twin B (e.g., 0= no difference in smoking behavior; 1= difference in smoking behavior). We did not need to transform the gender and rearing status variables since both twins of one pair had the same gender and rearing status. Twenty-one individuals were excluded from the pair level analysis since the data of their co-twin were missing due to death, refusal to participate or unknown contact information.

To test the effect of age at separation and family environment on DNA methylation, we ran multivariate linear regression analyses for each gene and the IRG individually within the

reared apart group. Again, the CpG sites served as dependent variables. We conducted an individual analysis for each of predictor (age at separation, conflict and cohesion) at a time. Additionally, we also controlled these analyses for gender, smoking behavior, alcohol use, and age at the time of blood collection.

All gene-wide statistical analyses were based on a significance level of $\alpha = .05$ (two tailed) and were run by using IBM SPSS Statistics version 25 for Windows. Due to multiple testing in the CpG sites analyses, we adjusted the significance level for these analyses according to Bonferroni correction. For analyses of OXT and AVP CpG sites, the corrected significance level was calculated to be $\alpha = .004$ (two tailed), and $\alpha = .0017$ (two tailed) for analyses of IGR CpG sites.

Results

Sample characteristics

As shown in Table 1, reared apart twins significantly differed from reared together twins in family conflict and the age at the time of separation. Twins that were reared apart were younger at the time of separation which corresponds to our cut-off age. Moreover, reared apart twins had a significantly smaller family conflict value which translates to a significantly more conflict-ridden family environment than that of reared together twins.

Table 1

Group differences in sample characteristics

Characteristics	reared apart (n = 58)	reared together (n = 91)	p-value
Sociodemographics			
Female sex (%)	34 (58.6%)	50 (54.9%)	.659
Age at the time of blood collection (SD)	68.6 (9.4)	67.3 (8.75)	.386
Age at the time of separation (SD)	2.99 (3.67)	19.89 (3.83)	< .001***
Current SES (SD)	-0.77 (3.22)	-0.14 (3.30)	.251
Health parameters			
Psychiatric history[a] (%)	9 (39.1%)	14 (50.0%)	.438
Alcohol use (%)	8 (13.8%)	21 (23.1%)	.163
Cancer diagnosis (%)	3 (5.2%)	2 (2.2%)	.326
Cardiovascular disease (%)	22 (40.7%)	32 (35.2%)	.731
Physical exercise (SD)	3.86 (1.00)	3.87 (1.12)	.974
BMI (SD)	26.31 (3.92)	27.48 (14.33)	.544
Family environment			
Conflict (SD)	18.00 (2.94)	18.93 (2.64)	.046*
Cohesion (SD)	18.20 (3.70)	19.04 (2.95)	.128

Note. [a] 35 individuals in the reared apart group and 62 individuals in the reared together group did not indicate whether they have a psychiatric history or not.

T-tests were conducted for continuous variables and Chi-Square tests for categorial variables. Significant findings are asterisked (* for $p < .05$; *** for $p < .001$).

Rearing status and DNA methylation

In Table 2, results of our CpG sites analyses on individual level are shown. Reared apart and reared together twins differ significantly in the methylation of one CpG site on AVP (cg04632887) before controlling for gender, age at the time of blood collection, smoking behavior, alcohol use and family environment. However, compared to the Bonferroni corrected significance level ($\alpha = .004$), this significant finding became insignificant.

Table 2

Methylation means per CpG site per group on individual level and p-values

	Means per group		p-values per model	
CpG site	reared apart (n = 58)	reared together (n = 91)	Uncontrolled model	Controlled model[a]
OXT				
cg04731988	-0.78 (0.61)	-0.76 (0.55)	.840	.720
cg07747220	0.40 (0.80)	0.26 (0.61)	.210	.647
cg16887334	1.14 (0.93)	1.11 (0.80)	.813	.467
cg13285174	0.16 (0.86)	0.09 (0.75)	.606	.681
cg26267561	0.28 (0.49)	0.27 (0.47)	.919	.574
cg01644611	0.83 (0.83)	0.78 (0.67)	.739	.370
cg13725599	1.30 (0.90)	1.36 (0.76)	.662	.853
cg19592472	-1.24 (0.65)	-1.32 (0.62)	.462	.329
cg26955850	-0.70 (0.45)	-0.62 (0.43)	.309	.184
cg09774842	-4.89 (0.35)	-4.84 (0.35)	.487	.443
cg06404175	-4.29 (0.19)	-4.32 (0.20)	.385	.529
cg12099952	-4.26 (0.44)	-4.29 (0.45)	.628	.653
IGR				
cg20245439	-0.45 (0.33)	-0.46 (0.33)	.733	.269
cg05844798	2.07 (0.40)	1.96 (0.42)	.121	.313
cg02872354	-3.52 (0.53)	-3.51 (0.49)	.912	.946
AVP				
cg14065127	-4.76 (0.55)	-4.86 (0.55)	.314	.321
cg11491381	-5.60 (0.26)	-5.61 (0.17)	.612	.137
cg25673357	-4.44 (0.27)	-4.43 (0.28)	.794	.492
cg03279206	-2.80 (0.66)	-2.87 (0.54)	.499	.700
cg04360210	-3.34 (0.53)	-3.26 (0.50)	.369	.320

cg25551168	-0.88 (0.25)	-0.87 (0.21)	.756	.121
cg16536918	-0.04 (0.29)	-0.11 (0.24)	.117	.483
cg24257309	-0.77 (0.29)	-0.77 (0.27)	.979	.524
cg05136169	0.61 (0.26)	0.56 (0.27)	.296	.596
cg02187522	0.35 (0.26)	0.33 (0.24)	.670	.938
➔cg04632887	-0.19 (0.35)	-0.31 (0.27)	.027*	.258
cg23169111	-0.29 (0.26)	-0.34 (0.22)	.184	.132

Note. Significant values are asterisked (*). Arrows (➔) were added to help to spot the significant results in the table. *n* represents the number of individuals in each group.

The standard deviation of the methylation means is given in brackets behind the methylation means. [a] Model was controlled for gender, age at the time of blood collection, smoking behavior, alcohol use and family environment.

Table 3 (below) displays the results for the group comparison on twin pair level. We compared mean intrapair differences in methylation between reared apart and reared together twin pairs. Significant group differences were found for cg26955850 (OXT) after controlling for gender, intrapair differences in age at the time of blood collection, intrapair differences in smoking behavior, intrapair differences in alcohol use and intrapair differences in family environment. Reared apart twin pairs exhibit greater mean intrapair differences in the methylation of this CpG site than reared together twin pairs. Again, compared to the Bonferroni corrected significance level ($\alpha = .004$), this significant finding became nonsignificant.

Table 3

Mean intrapair methylation differences per CpG site per group on twin pair level and p-values

	Mean intrapair differences per group		p-values per model	
CpG site	reared apart (n = 24)	reared together (n = 40)	Uncontrolled model	Controlled model[a]
OXT				
cg04731988	0.56 (0.36)	0.49 (0.51)	.566	.684
cg07747220	0.69 (0.37)	0.55 (0.55)	.276	.173
cg16887334	0.77 (0.51)	0.69 (0.50)	.547	.308
cg13285174	0.68 (0.41)	0.66 (0.60)	.872	.577
cg26267561	0.40 (0.28)	0.36 (0.39)	.617	.934
cg01644611	0.57 (0.37)	0.53 (0.54)	.328	.404
cg13725599	0.69 (0.51)	0.47 (0.45)	.713	.971
cg19592472	0.74 (0.46)	0.53 (0.61)	.067	.062
➔ cg26955850	0.46 (0.31)	0.43 (0.35)	.150	.046*
cg09774842	0.44 (0.34)	0.35 (0.34)	.760	.845
cg06404175	0.22 (0.12)	0.23 (0.19)	.801	.393
cg12099952	0.43 (0.39)	0.43 (0.36)	.982	.582
IGR				
cg20245439	0.38 (0.22)	0.39 (0.29)	.931	.316
cg05844798	0.33 (0.27)	0.47 (0.40)	.141	.126
cg02872354	0.69 (0.53)	0.53 (0.41)	.171	.154
AVP				
cg14065127	0.48 (0.47)	0.53 (0.51)	.725	.340
cg11491381	0.24 (0.22)	0.15 (0.14)	.056	.284
cg25673357	0.23 (0.22)	0.18 (0.13)	.306	.445
cg03279206	0.76 (0.55)	0.55 (0.41)	.084	.618
cg04360210	0.63 (0.43)	0.55 (0.38)	.405	.436
cg25551168	0.22 (0.22)	0.21 (0.18)	.894	.857
cg16536918	0.19 (0.16)	0.21 (0.16)	.674	.710
cg24257309	0.22 (0.22)	0.28 (0.24)	.319	.641
cg05136169	0.17 (0.17)	0.21 (0.18)	.371	.995
cg02187522	0.23 (0.15)	0.22 (0.18)	.839	.956
cg04632887	0.23 (0.17)	0.18 (0.15)	.177	.220
cg23169111	0.22 (0.20)	0.20 (0.20)	.625	.626

Note. Significant values are asterisked (*). Arrows (➔) were added to help to find the significant results in the table. *n* represents the number of twin pairs in each group.

The standard deviation of the mean intrapair differences is given in brackets behind the mean intrapair differences. [a] Model was controlled for gender, intrapair differences in age at the time of blood collection, intrapair differences in smoking behavior, intrapair differences in alcohol use and intrapair differences in family environment.

Predictors of DNA methylation within the reared apart group

Having looked at whether family environment and age at separation significantly predict OXT and AVP methylation, we found four significant CpG sites (Table 4). Family conflict significantly predicts the methylation of one OXT CpG site (cg26955850) and one AVP CpG site (cg02187522). Similarly, age at separation significantly predicts the methylation of one OXT CpG site (cg06404175) and one AVP CpG sites (cg11491381). As for our previous analyses, the results became nonsignificant after Bonferroni correction. No predictive effect of family cohesion was observed.

Table 2

Conflict, cohesion and age at the time of separation as predictors of methylation of single CpG sites within reared apart twins

	p-values per predictor		
CpG site	Family conflict	Family cohesion	Age at time of separation
OXT			
cg04731988	.394	.845	.370
cg07747220	.581	.985	.172
cg16887334	.592	.729	.320
cg13285174	.244	.749	.560
cg26267561	.259	.781	.432
cg01644611	.228	.907	.438
cg13725599	.578	.923	.470
cg19592472	.854	.542	.722
➔cg26955850	.040*	.228	.534

cg09774842	.368	.608	.571
➔cg06404175	.826	.105	.039*
cg12099952	.817	.790	.770
IGR			
cg20245439	.660	.605	.070
cg05844798	.863	.116	.972
cg02872354	.083	.999	.397
AVP			
cg14065127	.685	.291	.920
➔cg11491381	.244	.550	.046*
cg25673357	.970	.937	.481
cg03279206	.608	.766	.551
cg04360210	.495	.843	.136
cg25551168	.635	.848	.586
cg16536918	.513	.801	.220
cg24257309	.676	.679	.307
cg05136169	.302	.640	.206
➔cg02187522	.017*	.766	.233
cg04632887	.091	.548	.525
cg23169111	.120	.778	.868

Note. Model was controlled for gender, smoking behavior, alcohol use, and age at the time of blood collection. Arrows (➔) were added to help to find the significant results in the table. Significant results are asterisked (*).

To better spot methylation patterns, the relationships between pre-Bonferroni significant predictors and CpG sites are illustrated in Figure 1.

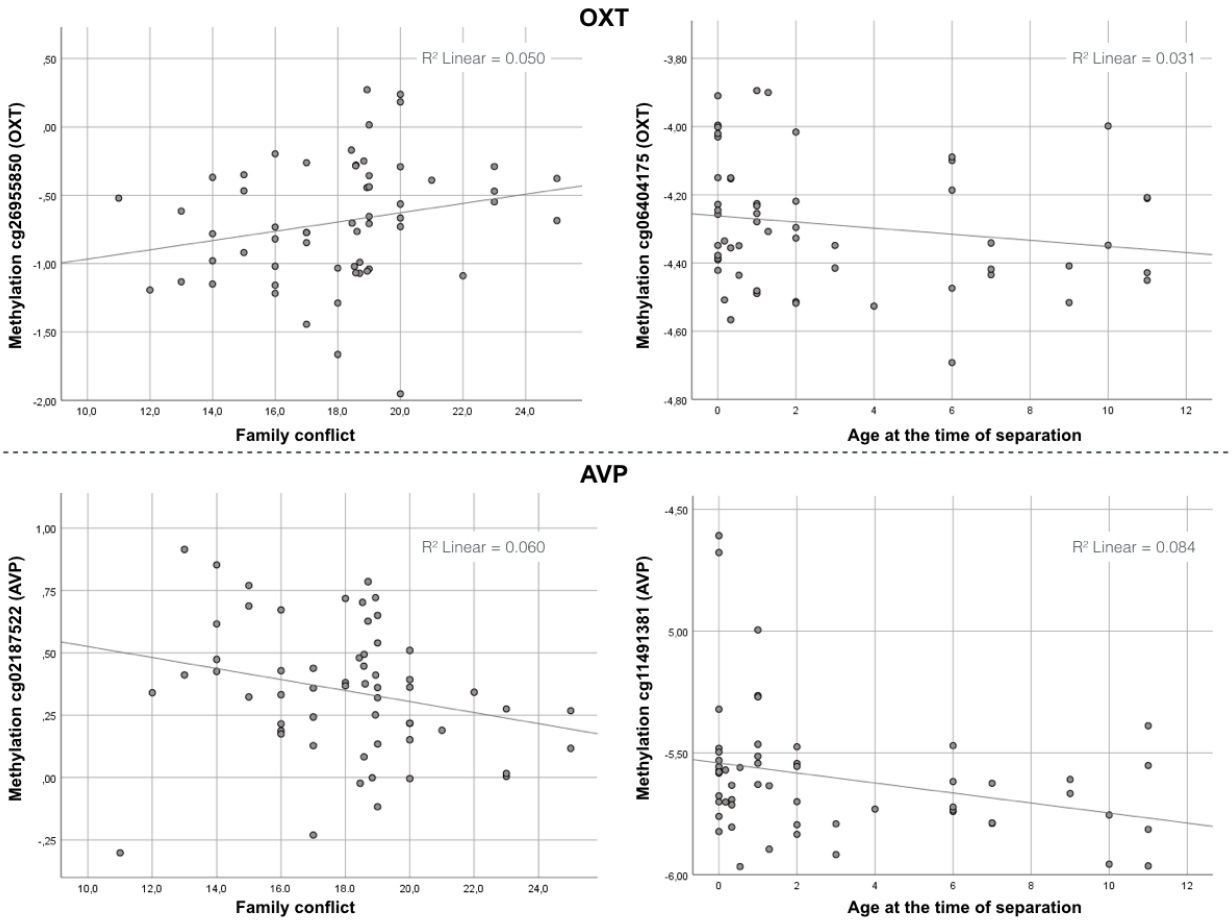

Figure 1. Graphical illustration of CpG sites that have a significant relationship with family conflict or age at the time of separation.

For both figures on the left, values on the X-axis represent the degree of family conflict: Low values mean high family conflict and vice versa. For both figures on the right, values on the X-axis represent the age at the time of separation in years. For all four figures, values on the Y-axis represent the degree of methylation of the respective CpG site.

Discussion

We investigated whether childhood separation impacts the methylation of OXT, AVP, and their IGR, whose expression affects the oxytocinergic and vasopressinergic systems which, in turn, are associated with psychosocial behavior and stress-regulation. We found significant relationships between childhood separation and the methylation of single CpG sites but no gene-wide effects. Furthermore, the methylation of single CpG sites within the reared-apart group was significantly predicted by family conflict and the age at the time of separation. After applying Bonferroni correction for multiple testing, these significances disappeared and thereby the support for our hypotheses. However, our pre-Bonferroni significant results are in line with previous research. Thus, nonsignificant effects in this study may be due to a small sample size and false negatives produced by the Bonferroni correction which easily inflates type II errors (Perneger, 1998). Hence, we discuss potential interpretations of our results in the light of previous research. Nevertheless, the lacking significance after correcting for multiple testing poses a caveat to this interpretation which makes further investigation indispensable.

As a general pattern, we observed that adversity in childhood (separation experience, young age at the time of separation, high family conflict) is connected to AVP hypermethylation, and OXT hypomethylation except for cg06404175. The tendency we found for OXT methylation is in line with the results by Sanwald et al. (2020), who reported a negative association between stressful life events and OXT methylation. This is unexpected considering that ELS causes hyperactivity of the HPA-axis, and OXT hypomethylation presumably leads to more oxytocin and thus soothes the HPA activity (Sanwald et al., 2020). Sanwald et al. (2020) suggest that the adversity-related OXT hypomethylation may be an adaptive reaction which aims at compensating the ELS-induced hyperactivity of the HPA-axis (Heim & Binder, 2012; Sanwald

et al., 2020). Then the inverse pattern of cg06404175 (OXT) methylation may hint at an age effect on this adaptive process. The younger the twins were at the time of their separation, the higher the methylation of cg06404175 (OXT).

Sanwald et al. (2020) only investigated OXT methylation. However, the contrary patterns in OXT and AVP methylation of our study further back Sanwald's et al. (2020) proposal of an ELS-related counterbalancing effect since AVP hypermethylation is thought to lead to less vasopressin and thereby a reduced anxiogenic impact (Murgatroyd et al., 2009). Hecker et al. (2016) observed a significant relationship between ELS and the methylation of cg03279206 (AVP). While we did not exactly replicate this finding, our results conform with those of Hecker et al. (2016) in the way that they too found AVP hypermethylation in relation to childhood adversity. Our findings and those of Sanwald et al. (2020) and Hecker et al. (2016) provide evidence that OXT and AVP methylation alterations following ELS are a resiliency rather than a vulnerability factor.

One CpG site that is particularly notable is cg26955850 (OXT) since its methylation was significantly related to both the rearing status and family conflict before the Bonferroni correction. Alike separation experiences, high family conflict also implies disruptions in relationships and hampers interpersonal closeness. Future research should specifically focus on the relationship of such disrupted attachment and the methylation of cg26955850 (OXT).

That we did not find a significant association between family cohesion and DNA methylation may have methodological or concept-inherent reasons. Our group comparisons of sample characteristics showed that reared apart and reared together twins did not significantly differ in family cohesion. This may be a real homogeneity or an indication of methodological flaws. In fact, the Cronbach's alpha of the cohesion scale is slightly lower than that of the

conflict scale. So, it is possible that the internal consistency of the family cohesion scale was insufficient for this study. However, there is also another possible explanation: For both separation and family conflict, emotionally stressful and negative events take place, while low family cohesion describes the absence of pleasant events. This inherent difference may impact DNA methylation and thereby our results.

Besides lacking significance after correcting for multiple testing and a possibly flawed cohesion scale, there is another, less decisive, limitation. There are no consistent standards in research that define the age period of childhood and early-life (Hambrick et al., 2019). Our operationalization of childhood separation as ELS is defined by a cut-off age that marks the beginning of puberty. Lacking consistencies among ELS-studies make it challenging to compare results. Moreover, potential effects of separation in later childhood on DNA methylation may not get detected. It is also possible that there is one or several sensitive age periods when individuals are especially vulnerable to separation experiences and sensitive to epigenetic changes. One known age period for the former is between six and twelve months (Varin et al., 1996). The present study was not able to investigate the significance of this early time for the relation of separation experiences and DNA methylation since there were too few cases in this particular age group. Since a possible resiliency mechanism of OXT methylation may not be present in very early childhood, we recommend future research to focus on these early stages of life. Thus, age at the time of separation should be kept being used as a continuous predictor aimed at identifying and investigating critical age phases. Possible non-linear relations between age at the time of separation and dependent variables such as DNA methylation should be considered.

Future research may also want to disentangle the separation effects caused by the separation from the co-twin and the separation from the primary caregiver. When reared apart,

both twins experienced the separation from a twin, but only one twin experiences the separation from both the co-twin and the primary caregiver(s). These different degrees of separation may have different effects on DNA methylation. In this study, we could not differentiate between those two degrees of separation due to missing data on which twin remained living with the primary caregivers after separation and which twin lived elsewhere. Hence, we are not able to know whether the effect of rearing status observed in our study was due to separation from the primary caregiver(s), co-twin, or from both of them.

Our study comprised a unique data set of a large number of monozygotic twins which allows studying environmental influences in a way that is not possible with any other sample. Although we did not find gene-wide effects and significant findings from our CpG sites analyses vanished after Bonferroni correction, our results concur with the findings from previous research and provide a direction for further investigation. To the best of our knowledge, this study was the first to examine the relationship between childhood adversity and the methylation of both OXT and AVP in humans, and thus lay the ground for future research on this topic.

References

Ahnert, L., Gunnar, M. R., Lamb, M. E., & Barthel, M. (2004). Transition to child care: Associations with infant–mother attachment, infant negative emotion, and cortisol elevations. *Child Development, 75*(3), 639–650. https://doi.org/10.1111/j.1467-8624.2004.00698.x

Baiden, P., Fallon, B., den Dunnen, W., & Boateng, G. O. (2015). The enduring effects of early-childhood adversities and troubled sleep among Canadian adults: a population-based study. *Sleep Medicine, 16*(6), 760-767. https://doi.org/10.1016/j.sleep.2015.02.527

Bird, A. P. (1986). CpG-rich islands and the function of DNA methylation. *Nature, 321*, 209–213. https://doi.org/10.1038/321209a0

Bowlby, J. (1978). *Attachment and loss, Vol. 2: Separation, anxiety and anger*. New York, US: Basic Books.

Boyd, C. P., Gullone, E., Needleman, G. L., & Burt, T. (1997). The Family Environment Scale: Reliability and normative data for an adolescent sample. *Family Process, 36*(4), 369–373. https://doi.org/10.1111/j.1545-5300.1997.00369.x

Carter, C. S. (2017). The oxytocin–vasopressin pathway in the context of love and fear. *Frontiers in Endocrinology, 8*, Article 356. https://doi.org/10.3389/fendo.2017.00356

Cochran, D., Fallon, D., Hill, M., & Frazier, J. A. (2013). The role of oxytocin in psychiatric disorders: a review of biological and therapeutic research findings. *Harvard Review of Psychiatry, 21*(5), 219–247. https://doi.org/10.1097/HRP.0b013e3182a75b7d

Corley, J., Cox, S. R., Harris, S. E., Hernandez, M. V., Maniega, S. M., Bastin, M. E., Wardlaw, J. M., Starr, J. M., Marioni, R. E., & Deary, I. J. (2019). Epigenetic signatures of smoking associate with cognitive function, brain structure, and mental and physical health

outcomes in the Lothian Birth Cohort 1936. *Translational Psychiatry, 9*(1), 248–248. https://doi.org/10.1038/s41398-019-0576-5

Cortes, L. R., Cisternas, C. D., & Forger, N. G. (2019). Does gender leave an epigenetic imprint on the brain? *Frontiers in Neuroscience, 13,* 173–173. https://doi.org/10.3389/fnins.2019.00173

Danese, A., & McEwen, B. S. (2012). Adverse childhood experiences, allostasis, allostatic load, and age-related disease. *Physiology & Behavior, 106*(1), 29–39. https://doi.org/10.1016/j.physbeh.2011.08.019

Ebstein, R., Israel, S., Lerer, E., Uzefovsky, F., Shalev, I., Gritsenko, I., Riebold, M., Salomon, S., & Yirmiya, N. (2009). Arginine vasopressin and oxytocin modulate human social behavior. *Annals of the New York Academy of Sciences, 1167*(1), 87–102. https://doi.org/10.1111/j.1749-6632.2009.04541.x

Ehlert, U. (2013). Enduring psychobiological effects of childhood adversity. *Psychoneuroendocrinology, 38*(9), 1850–1857. https://doi.org/10.1016/j.psyneuen.2013.06.007

Fields, R. L., House, S. B., & Gainer, H. (2003). Regulatory domains in the intergenic region of the oxytocin and vasopressin genes that control their hypothalamus-specific expression in vitro. *Journal of Neuroscience, 23*(21), 7801–7809. https://doi.org/10.1523/JNEUROSCI.23-21-07801.2003

Flynn, M., Cicchetti, D., & Rogosch, F. (2014). The prospective contribution of childhood maltreatment to low self-worth, low relationship quality, and symptomatology across adolescence: A developmental-organizational perspective. *Developmental Psychology, 50*(9), 2165–2175. https://doi.org/10.1037/a0037162

Foster, D., Davies, S., & Steele, H. (2003). The evacuation of British children during World War II: a preliminary investigation into the long-term psychological effects. *Aging & Mental Health, 7*(5), 398–408. https://doi.org/10.1080/1360786031000150711

Gainer, H., Fields, R. L., & House, S. B. (2001). Vasopressin gene expression: experimental models and strategies. *Experimental neurology, 171*(2), 190–199. https://doi.org/10.1006/exnr.2001.7769

Gimpl, G., & Fahrenholz, F. (2001). The oxytocin receptor system: structure, function, and regulation. *Physiological reviews, 81*(2), 629–683. https://doi.org/10.1152/physrev.2001.81.2.629

Goodman, J. B., Freeman, E. E., & Chalmers, K. A. (2019). The relationship between early life stress and working memory in adulthood: A systematic review and meta-analysis. *Memory, 27*(6), 868–880. https://doi.org/10.1080/09658211.2018.1561897

Green, J. G., McLaughlin, K. A., Berglund, P. A., Gruber, M. J., Sampson, N. A., Zaslavsky, A. M., & Kessler, R. C. (2010). Childhood adversities and adult psychiatric disorders in the national comorbidity survey replication I: associations with first onset of DSM-IV disorders. *Archives of General Psychiatry*, *67*(2), 113–123. https://doi.org/10.1001/archgenpsychiatry.2009.186.

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, 113*(27), 3816–3823. https://doi.org/10.1073/pnas.1602809113

Haine, R. A., Ayers, T. S., Sandler, I. N., & Wolchik, S. A. (2008). Evidence-based practices for parentally bereaved children and their families. *Professional Psychology: Research and Practice, 39*(2), 113–121. https://doi.org/10.1037/0735-7028.39.2.113

Hambrick, E. P., Brawner, T. W., & Perry, B. D. (2019). Timing of early-life stress and the development of brain-related capacities [10.3389/fnbeh.2019.00183]. *Frontiers in Behavioral Neuroscience, 13,* Article 183. https://doi.org/10.3389/fnbeh.2019.00183

Hecker, T., Radtke, K. M., Hermenau, K., Papassotiropoulos, A., & Elbert, T. (2016). Associations among child abuse, mental health, and epigenetic modifications in the proopiomelanocortin gene (POMC): A study with children in Tanzania. *Development and Psychopathology, 28*(4pt2), 1401–1412. https://doi.org/10.1017/S0954579415001248

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. https://doi.org/https://doi.org/10.1016/j.expneurol.2011.10.032

Heinrichs, M., von Dawans, B., & Domes, G. (2009). Oxytocin, vasopressin, and human social behavior. *Frontiers in Neuroendocrinology, 30*(4), 548–557. https://doi.org/10.1016/j.yfrne.2009.05.005

Herzberg, M. P., & Gunnar, M. R. (2020). Early life stress and brain function: Activity and connectivity associated with processing emotion and reward. *NeuroImage*, 209, Article 116493. https://doi.org/10.1016/j.neuroimage.2019.116493

Jansen, R. J., Tong, L., Argos, M., Jasmine, F., Rakibuz-Zaman, M., Sarwar, G., Islam, M. T., Shahriar, H., Islam, T., Rahman, M., Yunus, M., Kibriya, M. G., Baron, J. A., Ahsan, H., & Pierce, B. L. (2019). The effect of age on DNA methylation in whole blood among

Bangladeshi men and women. *BMC Genomics, 20*(1), 704–704. https://doi.org/10.1186/s12864-019-6039-9

Jiang, S., Postovit, L., Cattaneo, A., Binder, E. B., & Aitchison, K. J. (2019). Epigenetic modifications in stress response genes associated with childhood trauma. *Frontiers in Psychiatry, 10*, Article 808. https://doi.org/10.3389/fpsyt.2019.00808

Jones-Mason, K., Behrens, K. Y., & Gribneau Bahm, N. I. (2019). The psychobiological consequences of child separation at the border: lessons from research on attachment and emotion regulation. *Attachment & Human Development*, 1–36. https://doi.org/10.1080/14616734.2019.1692879

Kaufman, J., Plotsky, P. M., Nemeroff, C. B., & Charney, D. S. (2000). Effects of early adverse experiences on brain structure and function: clinical implications. *Biological Psychiatry, 48*(8), 778–790. https://doi.org/10.1016/S0006-3223(00)00998-7

King, L., Robins, S., Chen, G., Yerko, V., Zhou, Y., Nagy, C., Feeley, N., Gold, I., Hayton, B., & Turecki, G. (2017). Perinatal depression and DNA methylation of oxytocin-related genes: a study of mothers and their children. *Hormones and Behavior, 96*, 84–94. https://doi.org/10.1016/j.yhbeh.2017.09.006

Kompier, N. F., Keysers, C., Lucassen, P. J., Gazzola, V., & Krugers, H. J. (2019). Early life adversity and adult social behavior: focus on arginine vasopressin and oxytocin as potential mediators. *Frontiers in Behavioral Neuroscience, 13*, Article 143. https://doi.org/10.3389/fnbeh.2019.00143

Kraaijenvanger, E. J., Pollok, T. M., Monninger, M., Kaiser, A., Brandeis, D., Banaschewski, T., & Holz, N. E. (2020). Impact of early life adversities on human brain functioning: a

coordinate-based meta-analysis. *Neuroscience & Biobehavioral Reviews*, 113, 62–76https://doi.org/10.1016/j.neubiorev.2020.03.008

Loman, M. M., Wiik, K. L., Frenn, K. A., Pollak, S. D., & Gunnar, M. R. (2009). Postinstitutionalized children's development: growth, cognitive, and language outcomes. *Journal of Developmental and Behavioral Pediatrics, 30*(5), 426–434. https://doi.org/10.1097/DBP.0b013e3181b1fd08

Marazziti, D., & Dell'Osso, M. C. (2008). The role of oxytocin in neuropsychiatric disorders. *Current Medicinal Chemistry, 15*(7), 698–704. https://doi.org/10.2174/092986708783885291

Meinlschmidt, G., & Heim, C. (2007). Sensitivity to intranasal oxytocin in adult men with early parental separation. *Biological Psychiatry, 61*(9), 1109–1111. https://doi.org/10.1016/j.biopsych.2006.09.007

Meyer-Lindenberg, A., Domes, G., Kirsch, P., & Heinrichs, M. (2011). Oxytocin and vasopressin in the human brain: social neuropeptides for translational medicine [Report]. *Nature Reviews Neuroscience, 12(9)*, 524–538. http://doi.org/10.1038/nrn3044

Moore, L. D., Le, T., & Fan, G. (2013). DNA methylation and its basic function. *Neuropsychopharmacology, 38*(1), 23–38. https://doi.org/10.1038/npp.2012.112

Moos, R. H., & Moos, B. S. (2009). *Family environment scale manual: development, applications, research*. Center for Health Care Evaluation, Dept. of Veterans Affairs, Stanford University Medical Centers: Mind Garden.

Murgatroyd, C., Patchev, A. V., Wu, Y., Micale, V., Bockmühl, Y., Fischer, D., Holsboer, F., Wotjak, C. T., Almeida, O. F. X., & Spengler, D. (2009). Dynamic DNA methylation

programs persistent adverse effects of early-life stress. *Nature Neuroscience, 12*(12), 1559–1566. https://doi.org/10.1038/nn.2436

Murgatroyd, C., & Spengler, D. (2014). Polycomb binding precedes early-life stress responsive DNA methylation at the Avp enhancer. *PLoS ONE, 9*(3), e90277. https://doi.org/10.1371/journal.pone.0090277

Neumann, I. D., & Landgraf, R. (2012). Balance of brain oxytocin and vasopressin: implications for anxiety, depression, and social behaviors. *Trends in Neurosciences, 35*(11), 649–659. https://doi.org/10.1016/j.tins.2012.08.004

Pechtel, P., & Pizzagalli, D. A. (2011). Effects of early life stress on cognitive and affective function: an integrated review of human literature. *Psychopharmacology, 214*(1), 55–70. https://doi.org/10.1007/s00213-010-2009-2

Pedersen, N. L., McClearn, G. E., Plomin, R., Nesselroade, J. R., Berg, S., & DeFaire, U. (1991). The Swedish adoption twin study of aging: an update. *Acta Geneticae Medicae et Gemellologiae: Twin Research, 40*(1), 7–20. https://doi.org/10.1017/s0001566000006681

Perneger, T. V. (1998). What's wrong with Bonferroni adjustments. *BMJ (Clinical research ed.), 316*(7139), 1236–1238. https://doi.org/10.1136/bmj.316.7139.1236

Razin, A., & Cedar, H. (1991). DNA methylation and gene expression. *Microbiology and Molecular Biology Reviews, 55*(3), 451–458. PMCID: PMC372829

Sanwald, S., Gahr, M., Widenhorn-Müller, K., Schönfeldt-Lecuona, C., Richter, K., Connemann, B. J., Kammer, T., Montag, C., & Kiefer, M. (2020). Relation of promoter methylation of the oxytocin gene to stressful life events and depression severity. *Journal of Molecular Neuroscience, 70*(2), 201–211. https://doi.org/10.1007/s12031-019-01446-1

Stoop, R. (2012). Neuromodulation by oxytocin and vasopressin. *Neuron, 76*(1), 142–159. https://doi.org/10.1016/j.neuron.2012.09.025

UNICEF. (2019). Latest statistics and graphics on refugee and migrant children. https://www.unicef.org/eca/emergencies/latest-statistics-and-graphics-refugee-and-migrant-children

University of California Santa Cruz (2020). UCSC Genome Browser. https://genome.ucsc.edu/

Varin, D., Crugnola, C. R., Molina, P., & Ripamonti, C. (1996). Sensitive periods in the development of attachment and the age of entry into day care. *European Journal of Psychology of Education, 11*(2), 215–229. www.jstor.org/stable/23420064

Verhage, M. L., Schuengel, C., Madigan, S., Fearon, R. M., Oosterman, M., Cassibba, R., Bakermans-Kranenburg, M. J., & 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. https://doi.org/10.1037/bul0000038

Vinkers, C. H., Kalafateli, A. L., Rutten, B. P. F., Kas, M. J., Kaminsky, Z., Turner, J. D., & Boks, M. P. M. (2015). Traumatic stress and human DNA methylation: a critical review. *Epigenomics, 7*(4), 593–608. https://doi.org/10.2217/EPI.15.11

Wang, Y., Karlsson, R., Lampa, E., Zhang, Q., Hedman, Å. K., Almgren, M., Almqvist, C., McRae, A. F., Marioni, R. E., & Ingelsson, E. (2018). Epigenetic influences on aging: a longitudinal genome-wide methylation study in old Swedish twins. *Epigenetics, 13*(9), 975–987. https://doi.org/10.1080/15592294.2018.1526028

Weaver, I. C. G., Cervoni, N., Champagne, F. A., D'Alessio, A. C., Sharma, S., Seckl, J. R., Dymov, S., Szyf, M., & Meaney, M. J. (2004). Epigenetic programming by maternal behavior. *Nature Neuroscience, 7*(8), 847–854. https://doi.org/10.1038/nn1276

Young, L. J. (2001). Oxytocin and vasopressin as candidate genes for psychiatric disorders: lessons from animal models. *American Journal of Medical Genetics, 105*(1), 53–54.

Zahs, A., Curtis, B. J., Waldschmidt, T. J., Brown, L. A. S., Gauthier, T. W., Choudhry, M. A., Kovacs, E. J., & Bird, M. D. (2012). Alcohol and epigenetic changes: summary of the 2011 Alcohol and Immunology Research Interest Group (AIRIG) meeting. *Alcohol (Fayetteville, N.Y.), 46*(8), 783–787. https://doi.org/10.1016/j.alcohol.2012.05.005

Appendices

Appendix A. Comprehensive list of CpG sites analyzed in this study

Appendix B. Effects of rearing status (reared apart vs. reared together) on gene-wide OXT, AVP and IGR methylation

Appendix C. Effects of family environment on gene-wide OXT, AVP and IGR methylation within reared apart twins

Appendix D. Effects of age at the time of separation on gene-wide OXT, AVP and IGR methylation within reared apart twins

Appendix A

Comprehensive list of CpG sites analyzed in this study

Note. SNP = Single nucleotide polymorphism

CpG ID	Gene	SNP	CpG_relation
cg04731988	OXT	0	N_Shore
cg07747220	OXT	0	Island
cg16887334	OXT	0	Island
cg13285174	OXT	0	Island
cg26267561	OXT	0	Island
cg01644611	OXT	0	Island
cg13725599	OXT	0	Island
cg19592472	OXT	0	Island
cg26955850	OXT	0	Island
cg09774842	OXT	0	Island
cg06404175	OXT	0	Island
cg12099952	OXT	rs17339677	Island
cg20245439	IGR	0	S_Shore
cg05844798	IGR	0	N_Shore
cg02872354	IGR	0	Island
cg14065127	AVP	rs13041122	Island
cg11491381	AVP	0	Island
cg25673357	AVP	0	Island
cg03279206	AVP	0	Island
cg04360210	AVP	0	Island
cg25551168	AVP	0	S_Shore
cg16536918	AVP	0	S_Shore
cg24257309	AVP	0	S_Shore
cg05136169	AVP	0	S_Shore
cg02187522	AVP	0	S_Shore
cg04632887	AVP	0	S_Shore
cg23169111	AVP	0	S_Shore

Appendix B

Effects of rearing status (reared apart vs. reared together) on gene-wide OXT, AVP and IGR methylation

Model	F-value	p-value	Wilks' Λ	η2
Individual level				
OXT	1.38 (12,136)	.181	.89	.109
IGR	0.93 (3,145)	.427	.98	.019
AVP	0.95 (12,136)	.496	.92	.078
Individual level – controlled[a]				
OXT	0.39 (12,123)	.965	.96	.037
IGR	0.62 (3,132)	.603	.99	.014
AVP	0.91 (12,123)	.540	.92	.081
Pair level				
OXT	0.53 (12,51)	.887	.89	.110
IGR	1.45 (3,60)	.238	.93	.067
AVP	0.94 (12,51)	.519	.82	.181
Pair level – controlled[b]				
OXT	1.22 (12,44)	.300	.75	.250
IGR	1.70 (3,53)	.178	.91	.088
AVP	(12,44)	.855	.87	1.34

Note. [a] Model was controlled for gender, smoking behavior, alcohol use, age at the time of blood collection, family conflict and family cohesion. [b] Model was controlled for gender, intrapair differences in smoking behavior, intrapair differences in alcohol use, intrapair differences in age at the time of blood collection, intrapair differences in family conflict, and intrapair differences in family cohesion.

Appendix C

Effects of family environment on gene-wide OXT, AVP and IGR methylation within reared apart twins

Model	F-value	p-value	Wilks' Λ	η2
Family Conflict				
OXT	1.30	.250	.74	.258
IGR	1.43	.244	.93	.074
AVP	1.09	.392	.78	.255
Family Conflict - controlled[a]				
OXT	1.36	.285	.70	.295
IGR	1.23	.309	.92	.076
AVP	1.18	.335	.72	.282
Family Cohesion				
OXT	0.92	.532	.80	.198
IGR	0.90	.447	.95	.048
AVP	0.39	.959	.91	.095
Family Cohesion - controlled[b]				
OXT	0.56	.860	.84	.157
IGR	0.88	.461	.95	.055
AVP	0.29	.988	.91	.087

Note. [a] Model was controlled for gender, smoking behavior, alcohol use, age at the time of blood collection, age at the time of separation and family cohesion. [b] Model was controlled for gender, smoking behavior, alcohol use, age at the time of blood collection, age at the time of separation and family conflict.

Appendix D

Effects of age at the time of separation on gene-wide OXT, AVP and IGR methylation within

reared apart twins

Model	F-value	p-value	Wilks' Λ	η2
Uncontrolled				
OXT	0.69 (12,43)	.749	.84	.162
IGR	2.26 (3,52)	.093	.89	.115
AVP	1.11 (12,43)	.381	.76	.236
Controlled[a]				
OXT	0.76 (12,36)	.682	.80	.203
IGR	1.65 (3,45)	.192	.90	.099
AVP	1.31 (12,36)	.256	.70	.304

Note. [a] Model was controlled for gender, smoking behavior, alcohol use, age at the time of blood collection, family conflict and family cohesion.