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Relation of Promoter Methylation of the Oxytocin Gene to Stressful Life Events and Depression Severity

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Abstract

Oxytocin (OT) is a neuropeptide associated with trauma, sociality, and depression. Despite the widely accepted assumption of OT playing a role in the etiology of mood and anxiety disorders, associations between stressful life events, depression, and epigenetic regulation of the gene coding for OT (OXT) have not yet been investigated. We therefore aimed to examine the interrelations of stressful life events, depression severity, and methylation of the promoter region of OXT in a sample of $N = 146$ inpatients suffering from major depression. We found significant negative associations of stressful life events with mean methylation status as well as with methylation status of single CpG sites in the promoter region of OXT. There was no association between depression severity and OXT methylation. However, there were significant sex differences in methylation status of OXT with women showing higher methylation rates than men, putatively suggesting that in depression OXT is less activated in females compared to males. These results speak against an association of OXT methylation and depression severity, but support the assumption of a dysregulation of the OT system due to life stress. Our findings further emphasize the importance of including sex as an important factor in the investigation of the interrelations between OXT, stress, and depression.

Keywords Major depression · Oxytocin · Epigenetics · DNA methylation · Sex

Introduction

The neuropeptide oxytocin (OT) was originally known for its role in lactation (for a review, see Richard et al. 1991) and parturition (Russell et al. 2003). However, in humans, OT also acts as a neuromodulator in neural networks associated with trust (Kosfeld et al. 2005; for replication problems, see Nave et al. 2015), empathy (Decety and Batson 2009), and sociality (Jack et al. 2012). Therefore, it is a key element fostering the social bond between caretaker and infant as well as cohesion of families and social groups (Montag and Davis 2018; Waller et al. 2015). OT is synthesized as inactive precursor in the hypothalamus along with its carrier protein neurophysin I. The oxytocin-neurophysin I gene (OXT) is localized on human chromosome 20p13 (Rao et al. 1992).

Even though humans rely most strongly for their survival on social interactions and communication, they also have a history of within-species aggression, abuse, and warfare (Hrdy 2009). These are extreme forms of situations causing discomfort by shuttering an individual's sense of security or invulnerability to harm. Such stressful life events (SLEs) are assumed to trigger long-lasting to persistent hyper(re-)activity of the hypothalamic–pituitary–adrenal (HPA) axis via epigenetic mechanisms (McGowan et al. 2009; Murgatroyd et al. 2009; Roth et al. 2009). Persistent HPA hyper(re-)activity, which in turn increases stress vulnerability, has been identified as an important mechanism for the development of depression (Heim and Binder 2012; Heim et al. 2000). Depending on the nature of the stressor (e.g., psychosocial stress or physical experience), OT is released in stress-sensitive brain areas, thereby modulating the stress response (de Jong et al. 2015; Pierrehumbert et al. 2010; Winter and Jurek 2019). Also, the affective neuroscience theory by Panksepp (2004) suggests that in situations of sadness (caused by separation-distress),

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the administration of OT might be able to downregulate the activity of the SADNESS[1] circuitry. Even though the exact functional relevance of OT in HPA axis activity is not yet fully understood (Jurek et al. 2015), SLEs are associated with dysregulation of the endogenous OT system (Donadon et al. 2018) and are well-established major risk factors for the development of major depression (Culverhouse et al. 2018; Heim and Nemeroff 2001). SLEs may exert their pathogenic effect via epigenetic pathways (Szyf and Bick 2013).

Epigenetics is considered bridging the gap between genotype and phenotype. This term is used to describe changes in gene expression without changes in the underlying DNA sequence. An example of epigenetics is cell differentiation: Nearly all cells of a multicellular organism share one identical genotype. Nonetheless, a diversity of cell types with disparate, yet stable, profiles of gene expression and, therefore, distinct cell functions emerges during the development of an organism (Goldberg et al. 2007). However, a gene's activity may also be influenced by environmental signals, thus depending upon interindividual context (Weaver et al. 2004). Epigenetic regulation comprises mechanisms like DNA methylation, histone modifications, and noncoding RNAs. Probably, the most extensively investigated and characterized epigenetic biochemical modification of chromatin is DNA methylation (Feil and Fraga 2012). DNA methylation has been mainly examined at the 5′-position of cytosine residues of CpG dinucleotides. Genomic regions with a high density of CpG sites are referred to as CpG islands and can be found in the promoter regions of many genes (Bird 1986). DNA methylation can modify histone–DNA interactions, thus altering a gene's accessibility for transcription factors and thereby gene expression (Meaney and Szyf 2005). Methylation of CpG sites in the promoter region of a gene can for example prevent (but may also enhance) transcription factor binding and lead to a decline (or an increase) in transcription rate (Lim and Maher 2010; Watt and Molloy 1988). The role of DNA methylation in gene transcription inspired many scientists searching for potentially reversible biomarkers for disease risk and maintenance in humans (McGowan et al. 2009; Murgatroyd et al. 2009; Roth et al. 2009).

An early study focused on the examination of the glucocorticoid receptor gene (NR3C1) (Weaver et al. 2004) being closely associated with the HPA axis (Liu et al. 1997). In that study investigating the interaction of early life stress, DNA methylation, and HPA reactivity later in life, early life stress was operationalized as poor maternal care in a rat model (Weaver et al. 2004). Parenting in mammals is affected by hormones with OT playing a vital role (Feldman and Bakermans-Kranenburg 2017). OT is also associated with trauma (Donadon et al. 2018), HPA axis activity (Winter and Jurek 2019), and depressive-like behavior (Bosch and Young 2017; Jurek and Neumann 2018).

The focus of previous research regarding stress, DNA methylation, and depression has been on the oxytocin receptor gene (OXTR). Results of previous studies with regard to the association between exposure to prenatal stress and OXTR methylation were heterogeneous: One study found significant hypermethylation of the OXTR to be associated with maternal perinatal depressive symptoms (King et al. 2017). Another study reports that the total number of maternal adversities was negatively associated with OXTR methylation in cord blood (Unternaehrer et al. 2016). Last, there are studies reporting no significant association between prenatal exposure to maternal stress and OXTR methylation in cord blood (Rijlaarsdam et al. 2017) and between depressive symptoms in pregnancy and placental OXTR methylation (Galbally et al. 2018). On the other hand, early life adversity as well as persistent stressors has been shown to be associated with OXTR methylation (Gouin et al. 2017; Simons et al. 2017). Associations between methylation patterns of OXTR and depression diagnosis also differ across studies: Whereas two studies report hypermethylation of CpG sites in the OXTR (Bell et al. 2015; Chagnon et al. 2015), other studies found a negative association of OXTR methylation and depressive symptoms (Kimmel et al. 2016) as well as depression diagnosis (Reiner et al. 2015). However, the only two studies we are aware of that investigated OXT methylation in humans examined OXT methylation and sociability (Haas et al. 2016) as well as dynamic DNA methylation changes in mothers and postpartum maternal intrusiveness (Toepfer et al. 2019). In the first study, OXT methylation was negatively associated with a secure attachment style, the ability to recognize emotional facial expressions, and greater superior temporal sulcus activity during two social–cognitive functional MRI tasks (Haas et al. 2016). The second study found OXT methylation to decrease from early to mid-pregnancy and no further change until late pregnancy. Additionally, intrusive compared to non-intrusive mothers had 6% higher methylation of one CpG site in the OXT promoter in late pregnancy (Toepfer et al. 2019). The interrelations between SLEs, DNA methylation of OXT, and depression have not yet been examined.

Therefore, we examined the associations between DNA methylation of the promoter region of OXT and SLEs as well as current depressive symptoms in a sample of $N = 146$ inpatients suffering from major depression. Since there are considerable sex differences in stress response (Bale 2011; Bale and Epperson 2015), we also explored whether there are sex differences in OXT promoter methylation and whether sex interacts with stressful life events in the prediction of OXT promoter methylation. We further explored correlational patterns between SLEs, DNA methylation, and depression severity, controlling for other variables potentially confounding these associations, such as age, body mass index (BMI), current

[1] Written in capital letters in order to refer to the Panksepian nomenclature (Davis and Montag 2019).

medication, and substance use (Abraham and Fava 1999; de Wit et al. 2009; Dick et al. 2014; Ernst and Angst 1995; Feinberg et al. 2010; Lee and Pausova 2013; Philibert et al. 2012). Based on previous findings (Heim and Binder 2012; Heim and Nemeroff 2001), we assumed a positive association between SLEs and depression severity (see also the new Culverhouse et al. 2018 meta-analysis). Our overarching hypothesis for the association between OXT promoter methylation and SLEs or depression severity was that a higher number of SLEs is associated with high OXT methylation, presumably an indicator of lower transcription rate and therefore lower OT. This would coincide with the previously observed negative association between trauma and endogenous OT levels (Donadon et al. 2018). Since SLEs are positively associated with depression severity and presumably with OXT promoter methylation, one would assume OXT methylation and depression severity to be positively correlated as well. However, previous research showed an increased number of OXT-expressing neurons in the paraventricular nucleus in postmortem tissue of depressed patients (Purba et al. 1996) and an increase of OXT mRNA in melancholic type depressed patients (Meynen et al. 2007). Based thereon, low OXT methylation could also be associated with high depression severity. A previous study reported a correlation between SLEs and depression severity of $r = 0.24$ (Plieger et al. 2015). This indicates that there is a considerable proportion of unshared variance between the two variables. Consequentially, it is possible that there are diametrically opposed correlational patterns comparing the association between SLEs and OXT methylation to the association between OXT methylation and depression severity.

Patients and Methods

Participants

Data of $N = 146$ depressed inpatients (98 females) were collected. All participants were diagnosed for major depression (and no other mental illness) by a senior resident supervised by a psychiatrist at admission to the hospital using the Structured Clinical Interview for DSM-IV (American Psychiatric Association 2003). All participants were inpatients at the Department of Psychiatry at Ulm University and individually recruited. We calculated dose equivalents for antidepressants (weighted mean dose/fluoxetine 40 mg) (Hayasaka et al. 2015), received at the day of assessment. After completing the questionnaires described below, whole blood samples were taken and a standardized interview was conducted comprising a standardized semistructured inhouse questionnaire on sociodemographic variables and the Montgomery Asberg Depression Rating Scale (MADRS) to assess depression severity (Montgomery and Asberg 1979).

We also assessed the BMI measuring the height and weight of the participants. As a measure for substance use, we assessed frequency and dose of consumption as well as the kind of alcoholic drinks and caffeine containing products consumed in the reported frequency and dose. We then calculated grams per day for alcohol, cigarettes per day for nicotine, and milligrams per day for caffeine use. All participants provided written consent prior to participation (Table 1). The ethics committee of Ulm University, Ulm, Germany, approved the study.

Questionnaires

CLEQ

The Critical Life Events Questionnaire (CLEQ) assesses 30 potentially traumatic life events, such as experience of violence, natural disaster, man-made disaster, or death of a close person (Plieger et al. 2015). A weighted score was calculated adding up the product of the occurrence of each event and the experienced severity. If there were nine or more incompletely answered events, participants were excluded from further analysis with the CLEQ.

BDI-II

We also administered the Beck depression inventory (German version, BDI-II) to assess individual differences in the severity of depressive symptoms (Beck et al. 2006). The BDI-II consists of 21 items each assessing the current state of a symptom of depression with four given options (0–3). A total score is calculated adding up the scores of the 21 items. Higher scores indicate higher depression severity. Internal consistency was excellent with $\alpha = 0.91$.

Analyses of OXT Methylation

We selected the target region for methylation analysis based upon a previous study (Haas et al. 2016). Methylation status of the CpG-rich regions in the promoter of OXT (Fig. 1) was quantified by varionostic GmbH (Ulm, Germany) using the Sequenom Epityper MassArray System (San Diego, CA, USA). At first, genomic DNA from peripheral blood samples was automatically purified by means of the MagNA Pure® 96 system using a commercial extraction kit (MagNA Pure 96 DNA kit; Roche Diagnostics, Mannheim, Germany). Afterwards, genomic DNA was bisulfite treated. The bisulfite treatment and all steps of the EpiTYPER assay were performed under routine conditions as outlined in the manufacturer's suggested protocol. For the region of interest, amplicons were designed using Agena's EpiDESIGNER software (San Diego, CA, USA). These amplicons were PCR amplified using the following primers: forward

Table 1 Descriptive statistics of the examined variables

	n	Min	Max	Mean	SD
Age	146	18.00	65.00	39.08	14.35
Nicotine	146	0.00	24.00	4.97	7.24
Caffeine	146	0.00	650.00	99.51	139.51
Alcohol	136*	0.00	57.14	3.09	8.33
BMI	139*	16.02	42.45	25.75	5.79
Dose equivalents of antidepressants	116*	0.00	172.43	39.93	27.62
CLEQ	139*	0.00	112.00	22.88	19.94
MADRS	146	7.00	48.00	25.26	9.39
BDI	140*	10.00	59.00	32.28	11.13

Nicotine: cigarettes per day, caffeine: milligrams per day, alcohol: grams per day, BMI (body mass index): kg/m^2, antidepressants: dose equivalent to 40 mg fluoxetine

*Differing *n* reflect patients failing to complete all items of the inhouse questionnaire, CLEQ, or BDI-II or patients receiving medication not available in Hayasaka et al. (2015)

(aggaagagagGATTAGGGTTGG-GGATTTATTTTG) and reverse (cagtaatacgactcactatagggagaaggctACCTTCTATACCCAA-ACCATTAACC) corresponding to chr20:3,071,425–3,071,795.

In the next step, in vitro RNA transcription with subsequent base-specific cleavage using RNase A was performed resulting in fragmented RNA molecules. These RNA molecules may contain more than one CpG site. Cleavage products derived from methylated and unmethylated DNA are of identical length and differ only in their nucleotide composition due to bisulfite treatment. After sample conditioning, products were processed on a MALDI-TOF platform (Agena; MassARRAY 4). The different cleavage products created from methylated or unmethylated regions generated characteristic signal patterns that provided the basis for analysis by MALDI-TOF mass spectrometry. In analyzing the mass spectrum, the relative amount of methylation was calculated by comparing the difference in signal intensity between mass signals of the cleavage products and mass signals derived from completely methylated and unmethylated template DNA. Multiple CpG sites on one RNA molecule were analyzed as CpG unit.

Methylation status of the analyzed regions of the promoter OXT was examined with respect to single CpG sites or units as well as weighted (CpG units of two CpG sites were doubly weighted) mean methylation status across all 14 sites/units ($\alpha = 0.97$; mean DNA methylation: $M = 0.38$; $SD = 0.07$; $min = 0.20$; $max = 0.59$). Figure 2 presents boxplots of the methylation status for all examined CpG sites showing that there were no outliers or severe deviations from normality.

Statistical Analysis

Statistical analysis was conducted using R (R Development Core Team 2008). We computed partial Spearman's rank correlation coefficients (r_p) with sex, age, BMI, substance use (alcohol, nicotine, caffeine), and dose equivalents of antidepressants (weighted mean dose/fluoxetine 40 mg) (Hayasaka et al. 2015) as covariates to explore the association between the CLEQ score and depression severity (MADRS, BDI-II). We used the same approach to examine the associations of the methylation status of single CpG units in the promoter region of OXT and SLEs. Missing data was deleted pairwise. We used Spearman's rank correlation because some of the examined variables had outliers.

We additionally performed a hierarchical multiple regression analysis with mean methylation of OXT as dependent variable; age, BMI, substance use, and dose equivalents of antidepressants as covariates; and sex as well as CLEQ score as independent variables. In the next step, we added the interaction between sex and CLEQ score. Statistical significance was determined at $p < 0.05$.

Results

SLEs and Depression Severity

After controlling for sex, age, BMI, substance use, and dose equivalents of antidepressants, we found no significant correlation between the CLEQ score and depression severity according to the MADRS ($r_p = 0.17$, $p = 0.050$, one-tailed, n.s.). However, the CLEQ score was significantly positively correlated with depression severity assessed with the BDI-II ($r_p = 0.27$, $p = 0.003$, one-tailed). The result pattern did not change with separate analysis of the correlations in women and men ($r_{women} = 0.23$, $p = 0.036$; $r_{men} = 0.39$, $p = 0.024$).

Mean Methylation of the Promoter Region of OXT

There was no significant association between mean methylation status of OXT and depression severity, neither for

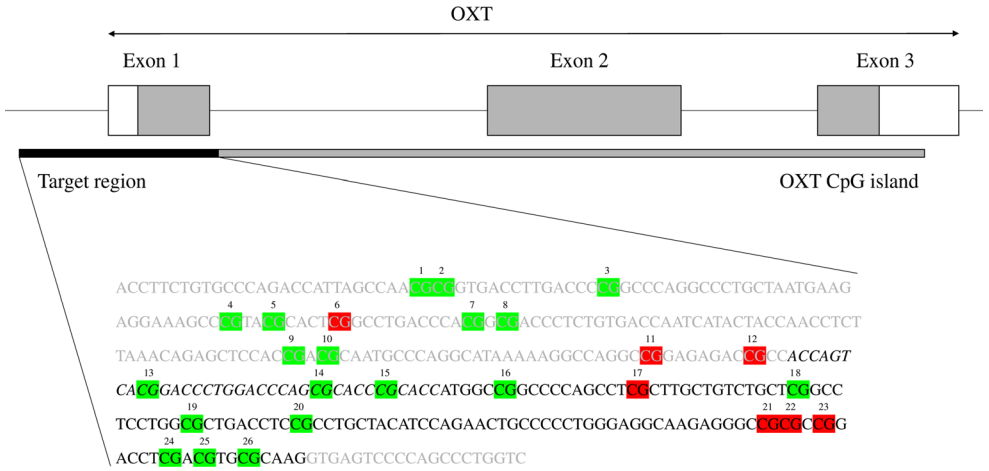

Fig. 1 CpG island and target region in OXT (chr20:3,071,425–3,071,795, hg38; CpG 1.2 corresponds to rs3,071,452–3,071,455). Light gray marks the region 5′ of the first exon of OXT. Black letters mark the first exon. Italic letters mark the untranslated region (UTR) within the first exon. Nonitalic letters mark the coding sequence (CDS) within the first exon. Methylation status of CpG sites marked in red was not analyzable. Haas et al. (2016) examined an averaged methylation score of CpG sites corresponding to CpG 1.2, 3, 4, 6, 11, 12, 15, 16, and 20. Toepfer et al. (2019) examined changes in the methylation status of one CpG site corresponding to CpG 5. CpG 1.2, 4, 5, 7.8, 9.10, and 14.15 were associated with SLEs in the whole sample and in men (for the latter only before controlling FDR). Further, CpG 29 was also associated with SLEs in the whole sample and in women (for the latter only before controlling FDR). CpG 19 and 24.25 were associated with depression in the whole sample but only before controlling FDR

MADRS (r_p = 0.02, p = 0.416, one-tailed) nor for BDI-II scores (r_p = 0.04, p = 0.330, one-tailed).

The regression model with age, BMI, substance use, and dose equivalents of antidepressants as covariates and sex as well as the CLEQ score as independent variables explained a significant amount of variance in mean OXT methylation (adjusted R^2 = 0.15, $F(8, 90) = 3.25$, p = 0.003). Sex and the CLEQ score were significant predictors of mean OXT methylation (Table 2). Females had significantly higher methylation status than males. Further, higher CLEQ scores indicating more stressful life events were associated with low mean OXT promoter methylation.

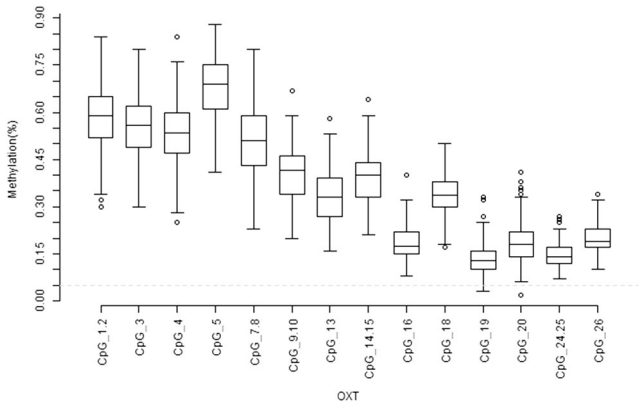

Fig. 2 Methylation status of the examined CpG units in the promoter region of OXT. Boxes cover methylation data between 25th and 75th quantile (median ± 1 interquartile range). Whiskers represent values falling within 1.5-fold the interquartile range. The dashed line represents the 5% detection limit for the Sequenom EpiTYPER platform. Methylation seemed to be lower within the CDS of exon 1

Adding the interaction of sex and CLEQ score to our model did not result in a significant increase in explained variance (adjusted R^2 = 0.17, $F(2, 86) = 0.13$, p = 0.71). Hence, there was no significant interaction of sex and CLEQ score in the prediction of mean OXT methylation ($b = -0.001$, SE = 0.001, $t(89) = -1.45$, p = 0.15).

Single CpG Sites

To further explore the association of OXT methylation and depression severity, we performed partial correlation analyses examining the association between the methylation of single CpG units in the OXT promoter and depression severity (as measured with the MADRS and the BDI-II) controlling for sex, age, BMI, substance use, and antidepressive medication. None of the single CpG sites or units was significantly associated with depression severity measured with the MADRS (partial Spearman's correlation coefficients ranged from $r_p = -0.03$ to r_p = 0.11, all p values > 0.05). There were two single CpG units significantly positively associated with BDI-II score: CpG 19 (r_p = 0.11, p = 0.026, one-tailed) and CpG 24.25 (r_p = 0.20, p = 0.040, one-tailed). However, after Benjamini–Hochberg correction (Benjamini and Hochberg 2000) controlling false discovery rate (FDR), none of these two correlations remained significant (p values > 0.300, one-tailed).

We did not find significant correlation coefficients between single CpG sites in the OXT promoter and depression severity (with age, BMI, substance use, and dose equivalents of antidepressants as covariates) looking at men and women separately, even before controlling FDR (results not shown).

We additionally performed partial correlation analyses for the association of the methylation of single CpG sites with CLEQ score. Before controlling FDR, eight of the 14 CpG units correlated significantly negative with the CLEQ score (Table 3). Even after controlling FDR, seven of these eight CpG units were significantly negatively correlated with critical life events. Correlation coefficients ranged from $r_p = -0.29$ to $r_p = 0.04$ and all but two correlation coefficients were negative.

When looking at correlations of single CpG sites in the OXT promoter and CLEQ score for men and women separately (with age, BMI, substance use, and dose equivalents of antidepressants as covariates), all but two correlation coefficients were negative (Table 4). In men, correlation coefficients were higher, but sample size was smaller than in women. After controlling FDR, none of the CpG units was significantly associated with the CLEQ score. This could be due to the smaller sample size.

Table 2 Regression coefficients for the prediction of mean OXT promoter methylation

Variable	*b*	SE	*t*	*p*
Intercept	*0.3970*	*0.0043*	*9.201*	*0.000*
Age	−0.0008	0.0005	−1.671	0.098
Nicotine	−0.0003	0.0009	−0.315	0.754
Caffeine	0.0000	0.0000	0.597	0.552
Alcohol	−0.0005	0.0008	−0.694	0.489
BMI	−0.0019	0.0014	−1.373	0.173
DE of antidepressants	0.0002	0.0002	0.755	0.452
Sex	*0.0410*	*0.0140*	*2.937*	*0.004*
CLEQ score	*−0.0008*	*0.0003*	*−2.353*	*0.021*

Numbers in italics correspond to significant predictors

DE, dose equivalents; *CLEQ*, Critical Life Events Questionnaire; *SE*, standard error

Discussion

We examined the associations between critical life events, depression severity, and methylation of the promoter region of OXT in a sample of $N = 146$ inpatients suffering from major depression. SLEs were significantly positively associated with depression severity as measured by means of the BDI-II. Contradictory to our hypothesis, we did not find an association of OXT promoter methylation and severity of depressive symptoms after controlling FDR. Nevertheless, OXT promoter methylation at two CpG units was significantly positively associated with depression severity measured with the BDI-II before controlling FDR. Furthermore, we observed a significant negative association between SLEs and mean OXT methylation. The negative association was also obtained for seven out of 14 single CpG units. The correlation coefficients between single CpG units and SLEs were negative for all but two CpG units. Sex and SLEs were significant predictors of mean OXT promoter methylation even after controlling for age, BMI, substance use, and antidepressive medication. Females showed a significantly higher methylation status of the OXT promoter.

OXT Methylation and Depression Severity

We did not observe a significant association between OXT promoter methylation and depression severity after controlling FDR. In accordance, a recent meta-analysis concluded that there is no association between the endogenous OT concentration and depression diagnosis (Engel et al. 2019b). However, since nonsignificance of this association after FDR correction could also be a result of our sample size, we briefly discuss the findings without FDR correction: Two CpG sites were significantly positively associated with depression severity as measured with the BDI-II. The associations' direction was contradictory to previous results (Meynen et al. 2007; Purba et al. 1996). However, provided that high OXT promoter methylation is associated with low transcription rate and finally with low brain oxytocin, our finding would fit theories postulating depression to be a shutdown mechanism to terminate protracted separation distress (Panksepp and Watt 2011; Watt and Panksepp 2009). This is also in line with a previous study reporting a negative association between plasma levels of oxytocin and depressive symptom severity (Scantamburlo et al. 2007). Further, there is considerable heterogeneity in the results regarding the association between the methylation status of the OXTR and depression. While some studies report a hypermethylation of the OXTR to be related to depression diagnosis (Bell et al. 2015; Chagnon et al. 2015), others found a negative association between OXTR methylation and depressive symptoms (Kimmel et al. 2016) as well as depression diagnosis (Reiner et al. 2015). This heterogeneity could be an artifact of sample composition and operationalization. While three studies focused on middle aged women (Bell et al. 2015; Kimmel et al. 2016; Reiner et al. 2015), one study investigated women aged 65 or older (Chagnon et al. 2015). Additionally, one study investigated depression and/or dysthymia (Reiner et al. 2015), one study focused on major depressive episodes as well as minor depression (Chagnon et al. 2015), and the other two studies examined postpartum depression (PPD) (Bell et al. 2015; Kimmel et al. 2016). However, it could also reflect the heterogeneity of depression itself: Patients suffering from depression often show different symptoms (even diametrically opposed in case of appetite and sleep). Perhaps, different subtypes of depression might be associated with a distinct etiology and with specific methylation patterns.

Sex and OXT Methylation

Sex and SLEs were significant predictors of mean OXT promoter methylation even after controlling for age, BMI,

Table 3 Partial Spearman's correlation coefficients for the association between CLEQ scores and methylation status of single CpG sites in the OXT promoter

OXT CpG	r_p	p	$p_{(BH)}$
CpG 1.2	*− 0.25*	*0.007*	*0.045*
CpG 3	−0.17	*0.046*	0.076
CpG 4	*− 0.22*	*0.014*	*0.045*
CpG 5	*− 0.21*	*0.016*	*0.045*
CpG 7.8	*− 0.20*	*0.025*	*0.050*
CpG 9.10	*− 0.20*	*0.022*	*0.050*
CpG 13	−0.14	0.089	0.113
CpG 14.15	*− 0.21*	*0.016*	*0.045*
CpG 16	−0.16	0.054	0.076
CpG 18	−0.16	0.052	0.076
CpG 19	0.04	0.351	0.378
CpG 20	*− 0.29*	*0.002*	*0.027*
CpG 24.25	0.01	0.462	0.462
CpG 26	−0.11	0.145	0.169

Covariates: sex, age, BMI, substance use (alcohol, nicotine, caffeine), and dose equivalents for antidepressants. One-tailed p values. Correlation coefficients in italics (with their respective p values) stayed significant after FDR correction

substance use, and antidepressive medication. Females showed a significantly higher methylation status of the OXT promoter. This observation is in line with the assumption of biological sex differences in brain functioning contributing to sex differences in the prevalence of major depression (Albert 2015). Supporting evidence for OXT playing a role in sex differences considering the prevalence of mood disorders stems from research postulating OT to be involved in the development of postpartum depression (Jobst et al. 2016; Lara-Cinisomo et al. 2017). In these studies, postpartum depressive symptoms were associated with lower levels of plasma OT (Jobst et al. 2016; Lara-Cinisomo et al. 2017). This is in line with our results of higher OXT promoter methylation status in women, since promoter methylation is often suggested being a mechanism of gene silencing (Jones and Takai 2001). Alternatively, sex differences in the OT system could be independent of the diagnosis of major depression.

Moreover, endogenous OT concentrations fluctuate together with concentrations of female sex hormones. A recent meta-analysis showed a significant increase of OT concentrations from the early follicular phase to ovulation and a significant decrease from ovulation to the mid-luteal phase (Engel et al. 2019a). Additionally, a recent study showed that OXT promoter methylation changed dynamically throughout pregnancy (Toepfer et al. 2019) rendering a menstrual cycle-dependent change in OXT methylation possible. However, it is, to the best of our knowledge, unknown how OXT promoter methylation is affected by female sex hormones and associated with immediate changes in endogenous OT concentrations. Further, it is unknown how epigenetic modifications of other genes playing a role for the endogenous OT system (e.g., the OXTR) interact in this regard. Nevertheless, there is a possibility that the observed sex difference in OXT promoter methylation is due to the fact that we did not control for phases of the menstrual cycle in women. Thus, the result of a sex difference in mean OXT methylation should be considered preliminary and needs further examination.

Table 4 Partial Spearman's correlation coefficients for the association between CLEQ scores and methylation status of single CpG sites in the OXT promoter separated by sex

OXT CpG	Men			Women		
	r_p	p	$p_{(BH)}$	r_p	p	$p_{(BH)}$
CpG_1.2	*− 0.38*	*0.024*	0.080	−0.19	0.065	0.282
CpG_3	−0.31	0.061	0.107	−0.14	0.142	0.282
CpG_4	*− 0.40*	*0.018*	0.080	−0.13	0.150	0.282
CpG_5	*− 0.37*	*0.029*	0.080	−0.14	0.141	0.282
CpG_7.8	*− 0.40*	*0.018*	0.080	−0.11	0.200	0.282
CpG_9.10	*− 0.34*	*0.043*	0.099	−0.15	0.125	0.282
CpG_13	−0.28	0.077	0.108	−0.06	0.309	0.309
CpG_14.15	−0.31	0.056	0.107	−0.15	0.113	0.282
CpG_16	*− 0.38*	*0.026*	0.080	−0.09	0.247	0.289
CpG_18	−0.24	0.116	0.147	−0.11	0.192	0.282
CpG_19	0.16	0.219	0.219	−0.08	0.276	0.297
CpG_20	−0.29	0.074	0.108	*− 0.30*	*0.008*	0.106
CpG_24.25	0.19	0.167	0.195	−0.11	0.201	0.282
CpG_26	−0.16	0.214	0.219	−0.09	0.242	0.289

Covariates: age, BMI, substance use (alcohol, nicotine, caffeine), and dose equivalents of antidepressants. One-tailed p values. Correlation coefficients in italics (with their respective p values) were significant before FDR correction

SLEs and OXT Methylation

Contradictory to our hypotheses, we found a significant negative association between SLEs and mean OXT methylation. The CLEQ score assesses stressors across the lifespan. Thus, the CLEQ score being associated with reduced OXT promoter methylation with respect to mean methylation as well as single CpG sites is in line with previous results showing significant associations between early life adversities as well as persistent adverse environments and OXTR methylation (Gouin et al. 2017; Simons et al. 2017). It is, however, worth mentioning that future investigations of stressors occurring early versus late in life could shed light on different OXT and OXTR methylation patterns depending on the timing of the stressor.

Our results are also in line with a recent review suggesting trauma to be associated with dysregulation of the endogenous OT system (Donadon et al. 2018). Even though trauma showed a moderate negative association with endogenous OT concentrations in this review, the suggested relationship

between trauma and OT is considered multifaceted and complex (Donadon et al. 2018). In line with this, OT can also promote "antisocial" behavior with its effects depending on various contextual and individual factors like gender or psychopathology (Shamay-Tsoory and Abu-Akel 2016). In accordance, increased OT has been associated with symptoms of social detachment (Munro et al. 2013). Trauma is assumed to promote social avoidance (Classen et al. 2001) and negative assumptions regarding interpersonal relationships (DePrince et al. 2009). OT is associated with agonistic tendencies in individuals chronically predisposed to perceive the social milieu as unsafe (Olff et al. 2013). Thus, it is possible that an interaction of SLEs and stress-induced low OXT promoter methylation is associated with a higher vulnerability for developing depressive disorders. In more detail, SLEs were associated with lower OXT methylation (and presumably higher OT) in our sample of depressed inpatients. Since trauma is also associated with perceiving social situations in negative terms (Classen et al. 2001; DePrince et al. 2009), the "antisocial" effects of OT may be triggered in individuals having low stress-induced OXT promoter methylation which may predispose these individuals to depression development. After all, depression often is associated with social withdrawal, feelings of inferiority, and social anxiety (Gilbert 2000). On the other hand, the negative association between OXT promoter methylation and SLEs could be an adaptive reaction counteracting trauma-induced hyper(re-)activity of the HPA axis (Heim et al. 2000). This hypothesis is congruent with the results of one study, which found lower OXT methylation to be associated with more secure attachment styles and an improved ability to recognize emotional facial expressions, both arguably features of human sociability (Haas et al. 2016). Insecure attachment styles are a potential mediator for the relationship between SLEs and depressive symptoms later in life (Hankin 2005). OT, on the other hand, can enhance the experience of attachment security (Buchheim et al. 2009). Thus, it would be an adaptive process to have higher OT-induced sociability and concomitantly recruit social resources after trauma exposure. After all, poor social support following trauma is among the greatest risk factors for the development of posttraumatic stress disorder (Ozer et al. 2003). However, these hypotheses should be tested using a longitudinal case–control design and comparison of populations suffering from different psychiatric disorders with respect to SLE-associated methylation patterns in the OXT promoter.

Limitations

There are some limitations that need to be considered when interpreting the results of the present study. First, we assessed methylation data by analyzing peripheral leukocytes possibly not providing a direct index of methylation status in the central nervous system. However, there are findings indicating high convergences between CpG island methylation levels across different tissues (Byun et al. 2009; Smith et al. 2015; Tylee et al. 2013). Second, although we investigated a relatively large number of CpG sites in our target region, our investigation is not exhaustive in terms of the CpG-rich region in OXT. However, we decided on investigating the only region that has been examined in previous studies in terms of DNA methylation (Haas et al. 2016; Toepfer et al. 2019). Third, we did not assess OXT mRNA or protein levels. Therefore, our study has no implications for OXT transcription. Lastly, data of a control group is missing so that any conclusions about the specificity of the findings with regard to depression are not warranted.

Conclusion

In conclusion, methylation status of two single CpG units was positively associated with depression severity before controlling FDR. Moreover, mean methylation as well as methylation status of single CpG sites in the OXT promoter was negatively associated with SLEs in a sample of depressed inpatients. These findings support the importance of the OT system in the development of or the resilience to psychopathology. In addition, there were sex differences in the epigenetic regulation of OXT promoting the assumed sex-specific effects of OT. This is important since sex-dependent regulation of the OT system could be relevant for the etiology and treatment of major depression. Therefore, future studies should examine the interrelations between SLEs, epigenetic regulation of OXT, and depression with respect to sex differences using a case–control design.

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Compliance with ethical standards

Conflict of Interest There authors declare that they have no competing interests.

Ethical Approval All procedures performed in this study were in accordance with the ethical standards of the ethics committee of Ulm University, Ulm, Germany (reference number: 25/18), and with the 1964 Helsinki Declaration and its later amendments.

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