European Neuropsychopharmacology (2019) 29, 147–155

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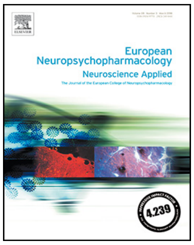

Oxytocin receptor gene methylation in male and female PTSD patients and trauma-exposed controls

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Received 10 November 2017; received in revised form 30 September 2018; accepted 23 October 2018

KEYWORDS
OXTR;
DNA-methylation;
PTSD;
Oxytocin;
Anhedonia;
Amygdala

Abstract

Oxytocin receptor gene (*OXTR*) DNA-methylation levels have been associated with trauma-exposure, mood- and anxiety disorders, and social processes relevant to posttraumatic stress disorder (PTSD). We hypothesized that *OXTR* methylation may play a role in the neurobiological underpinnings of PTSD. In the current study, we compared *OXTR* methylation between PTSD patients ($n = 31$, 14 females) and trauma-exposed controls ($n = 36$, 19 females). Additionally, the association between *OXTR* methylation and PTSD symptom severity and amygdala reactivity to an emotional faces task was assessed, as a neural hallmark of PTSD. DNA-methylation was investigated in the CpG island located at exon 3 of the *OXTR*, previously associated with *OXTR* expression. We observed a significant interaction between PTSD-status, sex and CpG-position on methylation levels. Post-hoc testing revealed that methylation levels at two specific CpG-sites were significantly higher in PTSD females compared to female trauma-exposed controls and PTSD males (CpGs Chr3:8809437, Chr3:8809413). No significant differences in methylation

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https://doi.org/10.1016/j.euroneuro.2018.10.006

were observed between male PTSD patients and controls. Furthermore, within PTSD females, methylation in these CpG-sites was positively associated with anhedonia symptoms and with left amygdala responses to negative emotional faces, although this was no longer significant after stringent correction for multiple-comparisons. Though the modest size of the current sample is an important limitation, we are the first to report on *OXTR* methylation in PTSD, replicating previously observed (sex-specific) associations of *OXTR* methylation with other psychiatric disorders.

1. Introduction

Post-traumatic stress disorder (PTSD) develops in about 10% of people that have been exposed to a traumatic event, such as a life-threatening accident, war or abuse. PTSD is characterized by intrusions of traumatic memories, avoidance of trauma-related cues, emotional numbing and negative affect, and hyperarousal (DSM-5). Heritability of PTSD is estimated between 30% and 50% (e.g. Stein et al., 2002) but environmental exposures play a major part; not only is trauma exposure a required precipitating factor for PTSD, but childhood adversity, life stress and social support also affect risk of PTSD development (Brewin et al., 2000). PTSD risk may depend on an interaction between genetic and environmental vulnerability factors, in which environmental contributing effects likely depend on genetic variation and vice versa (e.g. Wolf et al., 2014). Epigenetic processes such as DNA-methylation, which are responsive to factors such as stress and can alter gene-expression, have been found to mediate between trauma exposure and PTSD development (Klengel et al., 2014).

The oxytocin system may play a role in the (etio)pathology of PTSD, seeing its involvement in social reward functioning, fear and stress responses, which are affected in PTSD (Koch et al., 2014; Olff et al., 2010). For example, in the current sample of PTSD patients oxytocin administration was observed to increase reward pathway responses to social reward (Nawijn et al., 2016) and dampen amygdala reactivity to emotional faces (Koch et al., 2016a). Also, in the male PTSD patients in our sample we observed reduced endogenous oxytocin levels relative to trauma-exposed controls (Frijling et al., 2015). Increased methylation of the oxytocin receptor gene (*OXTR*) has been related to reduced *OXTR* expression (Gregory et al., 2009; Kusui et al., 2001) and reduced oxytocin blood levels (Dadds et al., 2013) though evidence has been mixed (Rubin et al., 2016). Sub-optimal functioning of the oxytocin system may reduce responsivity towards social reward or salient 'safety' cues in the environment, and the effectiveness of the oxytocin system to subsequently attenuate stress and fear responses and engage in prosocial approach-related behavior (Shamay-Tsoory and Abu-Akel, 2015). Thereby, OXTR methylation may play a role in the (etio)pathophysiology of PTSD. Indeed, OXTR methylation has been associated with biopsychosocial correlates of PTSD, such as responses to socio-affective stimuli (Haas et al., 2016; Rubin et al., 2016) which may be associated with reduced processing of social reward cues observed in PTSD (Nawijn et al., 2015). OXTR methylation was also associated with stress responsivity (Unternaehrer et al., 2012) and amygdala reactivity (Puglia et al., 2015; Ziegler et al., 2015), which could underlie enhanced fear responses and altered stress recovery observed in PTSD (Koch et al., 2014). OXTR methylation may also be affected by trauma exposure. Increased OXTR methylation was previously observed in individuals with childhood adversity (Gouin et al., 2017; Smearman et al., 2016; Unternaehrer et al., 2015), and interacted with childhood abuse in association with adult psychopathology (Gouin et al., 2017; Smearman et al., 2016). Furthermore, *OXTR* methylation has been associated with presence of mood and anxiety disorders (Bell et al., 2015; Cappi et al., 2016; Chagnon et al., 2015; Kimmel et al., 2016; Reiner et al., 2015; Smearman et al., 2016; Ziegler et al., 2015). Thus, higher levels of OXTR methylation may exist in patients with PTSD, which may be associated with biopsychosocial correlates of PTSD, such as reduced sensitivity to social reward and increased fear and stress responsivity.

Sex differences are present in PTSD prevalence and (etio)pathophysiological mechanisms, as well as oxytocin system functioning. PTSD is more prevalent in women, thought to relate in part to differences in psychobiological responses to stress (Pineles et al., 2017), such as sex differences in DNA methylation (Uddin et al., 2013). Effects of oxytocin administration are also moderated by sex (e.g. Koch et al., 2016b) and *OXTR* gene polymorphisms have been observed to affect stress-responses in a sex-dependent manner (Love et al., 2012). Female-specific associations between OXTR methylation and psychopathology, early life adversity and socio-affective functioning have been reported (Gouin et al., 2017; Rubin et al., 2016). Therefore, we included male and female participants to investigate sex-specific associations.

Based on these findings, *OXTR* DNA-methylation may be associated with PTSD and its neurobiological correlates. Therefore, we explored the association between *OXTR* methylation and PTSD in a sample of trauma-exposed police-officers with and without PTSD, hypothesizing increased methylation in PTSD patients compared to controls. Further knowledge of the relation between *OXTR* methylation and PTSD may provide valuable insights in the neurobiological underpinnings of PTSD psychopathology.

2. Experimental procedures

2.1. Sample

The current study was part of a larger neuroimaging protocol, investigating the effects of a single intranasal oxytocin

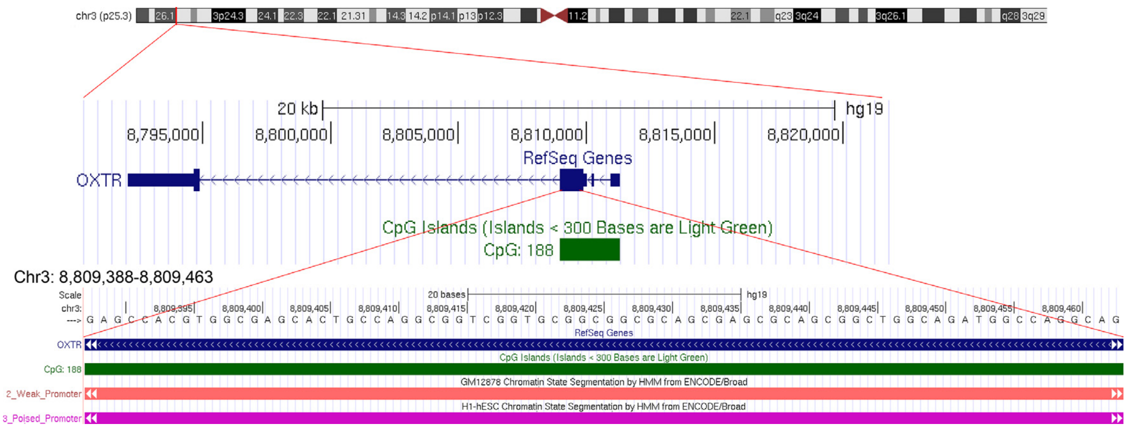

Fig. 1 Schematic representation of the *OXTR* gene. *OXTR* is located on chromosome 3 (p25.3) and consists of four exons. The translation start site is located in exon 3, the stop codon in exon 4. The target sequence is shown enlarged (Chr3:8809388–8809463, not to scale, UCSC human genome browser GRCh37/hg19) and was located at the protein-coding region of exon 3, part of a CpG-island spanning exon 1–3.

administration on neural processing in police officers with and without PTSD (Koch et al., 2016a). Thirty-one PTSD patients (14 females) and 36 controls (19 females) were considered for *OXTR* analyses. PTSD patients fulfilled DSM-IV criteria for current PTSD and had a Clinician Administered PTSD Scale (CAPS) score $\geq$ 45. Controls reported at least one traumatic event and had a CAPS score $<$ 15, no lifetime MDD or PTSD, or current psychiatric disorders. All participants were free of daily use of psychotropic medication. For details see Koch et al. (2016a).

2.2. *OXTR* quantitative DNA-methylation analysis

2.2.1. Sample preparation

DNA was isolated from whole blood. DNA-samples were randomized followed by bisulphite conversion using the EZ DNA Methylation^{TM} of Zymo Research, according to manufacturer's protocol. Tested regions located in *OXTR* were selected based on previous research (Unternaehrer et al., 2012; Ziegler et al., 2015). Methylation within this CpG-island in exon 3 has been associated with affective disorders and stress responses (Cappi et al., 2016; Chagnon et al., 2015; Smearman et al., 2016; Unternaehrer et al., 2016, 2015, 2012; Ziegler et al., 2015). We investigated 12 CpG-sites located between Chr3:8809388–8809463 (hg19) in the protein-coding region of *OXTR* exon 3 (Fig. 1, Tables S1, S2). Prepared amplicons were submitted for sequencing applying the Illumina Miseq 300V2 kit. For additional information, see supplemental methods and Krzyzewska et al. (2018).

2.2.2. Quality control of Miseq Illumina (QC)

Sequencing run quality was regarded successful if 70% of bases had a quality score $\geq$ Q30 (Quality Scores for Next-Generation Sequencing, Illumina). For QC of methylation status, we used 100% and 0% methylated DNA references (QIAGEN), prepared in the same way as participant samples. Sequences were aligned using Genomic Viewer software (version2.3.57). The threshold for minimal coverage was 150 reads per single CpG-site per subject. Single CpG-sites with missing data for more than 30% of subjects were excluded from further analysis. For comparability, we retained the CpG numbering as described by Ziegler et al. (2015).

2.3. Imaging data

Participants completed two scanning sessions. Prior to scanning, intranasal oxytocin or placebo was administered in a double-blind cross-over design. Here, we only included data collected under placebo. Amygdala reactivity was assessed using an emotional face matching task, contrasting angry-fearful faces relative to neutral-happy faces, in which we previously observed deficits in PTSD patients (Koch et al., 2016a). For more details see supplemental methods and Koch et al. (2016a).

2.4. Statistical analyses

Analyses were conducted in SPSS v24. Group differences in sample characteristics were investigated separately in males and females using independent sample *t*-tests, Mann-Whitney *U* tests and Fisher's exact tests. Associations between methylation in all single CpG-sites and potential confounders were investigated using correlation analyses (continuous variables age, years of service, work-related and childhood-trauma exposure, body-mass index (BMI), estimated relative blood cell counts based on 450 K DNA-methylation array, see supplemental methods), independent *t*-tests and ANOVAs (categorical variables education, smoking, hormonal contraceptives, comorbid MDD). Variables that were significantly associated with methylation were included as covariates in subsequent analyses.

2.4.1. OXTR methylation and PTSD-status

Methylation levels were highly correlated between CpG-sites (Table S4). To optimally deal with multicollinearity and missing data, we performed linear mixed modeling

(LMM), as previously employed in *OXTR* methylation analyses (Reiner et al., 2015; Unternaehrer et al., 2015). Main and interaction effects of PTSD-status, sex and CpG-position on *OXTR* methylation were investigated (see supplemental information for details). Post-hoc analyses were run using LMM and independent *t*-tests (see supplemental information for details). *P*-values were corrected for multiple comparisons using Benjamin–Hochberg's false discovery rate (FDR)-correction. Subsequently, we ran the above-stated model again, while including potential confounders (i.e. variables significantly associated with methylation) as covariates, to verify that our main outcomes did not alter. Also, sensitivity analyses were conducted by excluding PTSD patients with comorbid MDD to control for possible effects of MDD. Effect sizes were calculated using Cohen's *d*.

2.4.2. OXTR methylation, PTSD symptoms and amygdala reactivity

Methylation levels in CpG-sites that were significantly associated with PTSD were further investigated in relation to clinical and biological correlates of PTSD. Within PTSD patients, we correlated methylation levels of PTSD-associated CpG-sites with total PTSD symptom severity (total CAPS score), and symptom severity on the re-experiencing, avoidance, anhedonia/emotional numbing and hyperarousal symptom-scales, according to the 4-factor model of PTSD. Furthermore, we correlated methylation levels to amygdala responses to emotional faces. Significant effects were re-run using partial correlations while including potential confounders and comorbid MDD as covariates.

3. Results

3.1. Sample

There were no significant differences between patients and controls on any of the demographic or biological characteristics ($p > 0.1$, Table S3). Importantly, patients and controls did not differ significantly in exposure to number of types of work-related or childhood traumatic events (Table S3).

3.2. Participant characteristics and *OXTR* methylation

Similar to previous *OXTR* methylation studies (Reiner et al., 2015; Unternaehrer et al., 2012; Ziegler et al., 2015), low coverage particularly at the 3′ end of the target sequence led to missing data (Table S2). Sites with missing data in > 30% of participants were excluded (CpG1, CpG2, CpG5, CpG6), leaving a total of eight CpG-sites for statistical analyses (CpG3, CpG4, CpG7, CpG8, CpG9, CpG10, CpG11, CpG12). Methylation levels showed strong and significant positive correlations between CpG-sites ($r = 0.826$-0.980, all $p < 0.001$, Table S4).

Age was significantly negatively correlated with methylation in CpG4 ($r = -0.284$, $p = 0.024$). Years of service was significantly correlated with methylation levels in CpG3 ($r = -0.294$, $p = 0.047$) and CpG4 ($r = -0.268$, $p = 0.036$), though no longer significant when controlling for age ($p > 0.1$). Monocyte cell count was significantly negatively correlated with methylation in several CpG-sites (CpG8, $r = -0.288$, $p = 0.024$; CpG9, $r = -0.281$, $p = 0.030$; CpG10, $r = -0.325$, $p = 0.013$; CpG12, $r = -0.270$, $p = 0.043$). Natural killer cell count was negatively correlated with methylation in CpG10 ($r = -0.264$, $p = 0.045$). Therefore, age, monocyte and natural killer cell count were included as covariates in subsequent analyses. No other potential confounders were significantly associated with methylation in any of the CpG-sites ($p > 0.05$).

3.3. Effects of PTSD-status and sex on *OXTR* methylation

When investigating *OXTR* methylation using LMM, PTSD-status had no significant main effect on overall methylation ($F(1,66.846) = 1.287$, $p = 0.261$). There was a marginal overall effect of sex ($F(1, 66.846) = 3.038$, $p = 0.086$). No significant interaction effect between PTSD-status and sex on methylation was observed ($F(1,66.846) = 2.521$, $p = 0.117$). CpG-position had a strong significant main effect on methylation, confirming that methylation levels differed between single CpG-sites ($F(7,64.261) = 249.082$, $p < 0.001$). Furthermore, we observed a significant interaction between CpG-position and PTSD-status ($F(7,64.261) = 3.565$, $p = 0.003$), CpG-position and sex ($F(7,64.261) = 2.805$, $p = 0.013$) and CpG-position, PTSD-status and sex ($F(7,64.261) = 4.681$, $p < 0.001$), indicating that the effects of PTSD-status and sex on methylation levels were dependent on CpG-position. All significant effects remained significant when including covariates in the model, and when excluding PTSD patients with comorbid MDD from the model ($p < 0.05$).

To investigate whether the effect of PTSD on methylation of single CpG-sites was present in males or females, we ran stratified post-hoc LMMs to test the main and interaction effects of PTSD and CpG-position on OXTR methylation, separately within males and females (see supplemental methods for details). There was a significant interaction effect between CpG-position and PTSD-status in females ($F(7,29.700) = 7.494$, $p < 0.001$), and in males ($F(7,31.992) = 2.540$, $p = 0.034$), both remained significant after FDR-correction. The PTSD by CpG-position interaction in females remained significant after correction for covariates ($p < 0.001$) and in sensitivity analyses after exclusion of PTSD females with comorbid MDD ($p < 0.001$), but the interaction in males became non-significant after correction for covariates ($p = 0.060$) and exclusion of comorbid MDD ($p = 0.066$). Further post-hoc testing in females demonstrated a significant hypermethylation in PTSD in CpG3 ($F(1,32.39) = 7.493$, $p = 0.010$, Cohen's $d = -0.86$), CpG7 ($F(1,32.95) = 4.622$, $p = 0.039$, Cohen's $d = -0.67$), CpG9 ($F(1,33.00) = 9.796$, $p = 0.004$, Cohen's $d = -1.04$), and CpG12 ($F(1,33.00) = 4.604$, $p = 0.039$, Cohen's $d = -0.67$) (Fig. 2). The effect in CpG3 and CpG9 survived FDR-correction, and correction for covariates (CpG3 $p = 0.011$, CpG9 $p = 0.007$) and comorbid MDD (CpG3 $p = 0.022$, CpG9 $p = 0.015$). Post-hoc testing in males demonstrated that PTSD was not significantly associated with methylation in any of the CpGs ($p > 0.10$).

Exploratory post-hoc LMMs testing the effects of sex and CpG-position stratified for PTSD-status further showed

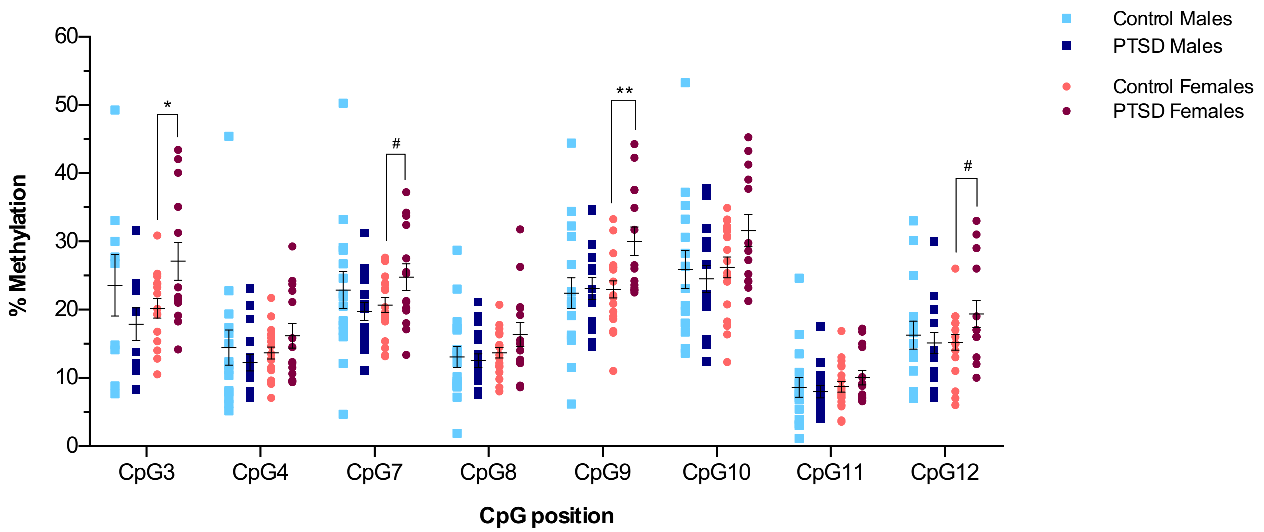

Fig. 2 Dispersion plot of methylation levels (%) per CpG-site per participant group. Means and error bars (±1SE) are displayed per group. # = $p < 0.05$ but not significant after FDR-correction, $^{*} = p < 0.05$, $^{**} = p < 0.01$.

significant hypermethylation in PTSD females in CpG3 ($p = 0.007$) and CpG9 ($p = 0.006$) compared to PTSD males (see supplemental information for detailed results). Within controls, no sex differences in methylation were observed at any of the CpG-positions.

3.4. Correlation between *OXTR* methylation, PTSD symptoms and amygdala responses

Within PTSD females, CpG3 and CpG9 methylation was significantly positively correlated with the anhedonia subscale (CpG3 $r = 0.658$, $p = 0.014$, Cohen's $d = 1.30$; CpG9 $r = 0.546$, $p = 0.044$, Cohen's $d = 1.70$, Fig. 3A, B, Supplemental Table S5), but not with other subscales or total symptom severity ($p > 0.10$). The associations between anhedonia and CpG3 and CpG9 methylation remained (marginally) significant after correction for covariates (CpG3 $r = 0.815$, $p = 0.026$; CpG9 $r = 0.707$, $p = 0.075$) and after correcting for comorbid MDD (CpG3, $r = 0.603$, $p = 0.038$; CpG9, $r = 0.610$, $p = 0.035$). However, they were no longer significant after FDR-correction. Additional exploratory correlation analyses were carried out in male PTSD patients, suggesting similar direction (Fishers r-to-Z $p < 0.10$) but non-significant associations in males (Supplementary results, Supplemental Table S5).

Explorative correlation analyses showed that CpG3 and CpG9 methylation in PTSD females were marginally negatively correlated with left amygdala responses towards negative faces (CpG3, $r = -0.613$, $p = 0.059$, Cohen's $d = -1.55$; CpG9, $r = -0.587$, $p = 0.058$, Cohen's $d = -1.45$; Fig. 3C, D). In control females, (marginally) significant positive correlations were observed (CpG3 $r = 0.466$, $p = 0.093$, Cohen's $d = 1.05$; CpG9 $r = 0.481$, $p = 0.044$, Cohen's $d = 1.10$; Fig. 3C, D), though these were no longer significant after FDR-correction. Fishers r-to-z transformation-tests showed the correlations between CpG3 and CpG9 methylation and amygdala responses significantly differed between female PTSD patients and controls (CpG3 $Z = -2.52$, $p = 0.012$, Cohen's $d = -1.20$; CpG9 $Z = -2.73$, $p = 0.006$, Cohen's $d = -1.18$).

4. Discussion

In the current study, we report the first investigation of *OXTR* DNA-methylation in a sample of PTSD patients and trauma-exposed healthy controls. We observed two *OXTR* CpG-sites in exon 3 that were significantly hypermethylated in female PTSD patients relative to female controls and PTSD males. Within female PTSD patients, high methylation at these two *OXTR* CpG-sites was associated with high anhedonia symptoms and low amygdala differentiation between negative relative to positive facial expressions with large effect sizes, although the significance for these results did not survive stringent correction for multiple comparisons. These findings were also significantly present when controlling for or excluding patients with comorbid MDD. In males, PTSD-status was not significantly associated with *OXTR* methylation.

These findings are in line with previous reports of *OXTR* hypermethylation in various psychiatric patient groups in the same CpG island (exons 1-3). Most studies investigated CpGs in either exon 3 (as in the current study) or in exon/intron 1 (for overview see Maud et al., 2018). Considering the low correlations observed in methylation levels between CpGs located in exon 3 and in exon/intron 1 (Smearman et al., 2016), we only consider studies investigating CpGs in exon 3 for comparison with the current findings. Higher exon 3 *OXTR* methylation, as we observed in PTSD females, was also observed in females with depression and anxiety disorders (Chagnon et al., 2015), and in males and females with obsessive compulsive disorder relative to controls (Cappi et al., 2016). It is however in contrast with reports of lower exon 3 *OXTR* methylation in a mixed-gender sample of social anxiety disorder (SAD) patients (Ziegler et al., 2015) and lack of association between exon 3 methylation and anxiety and depression symptoms in

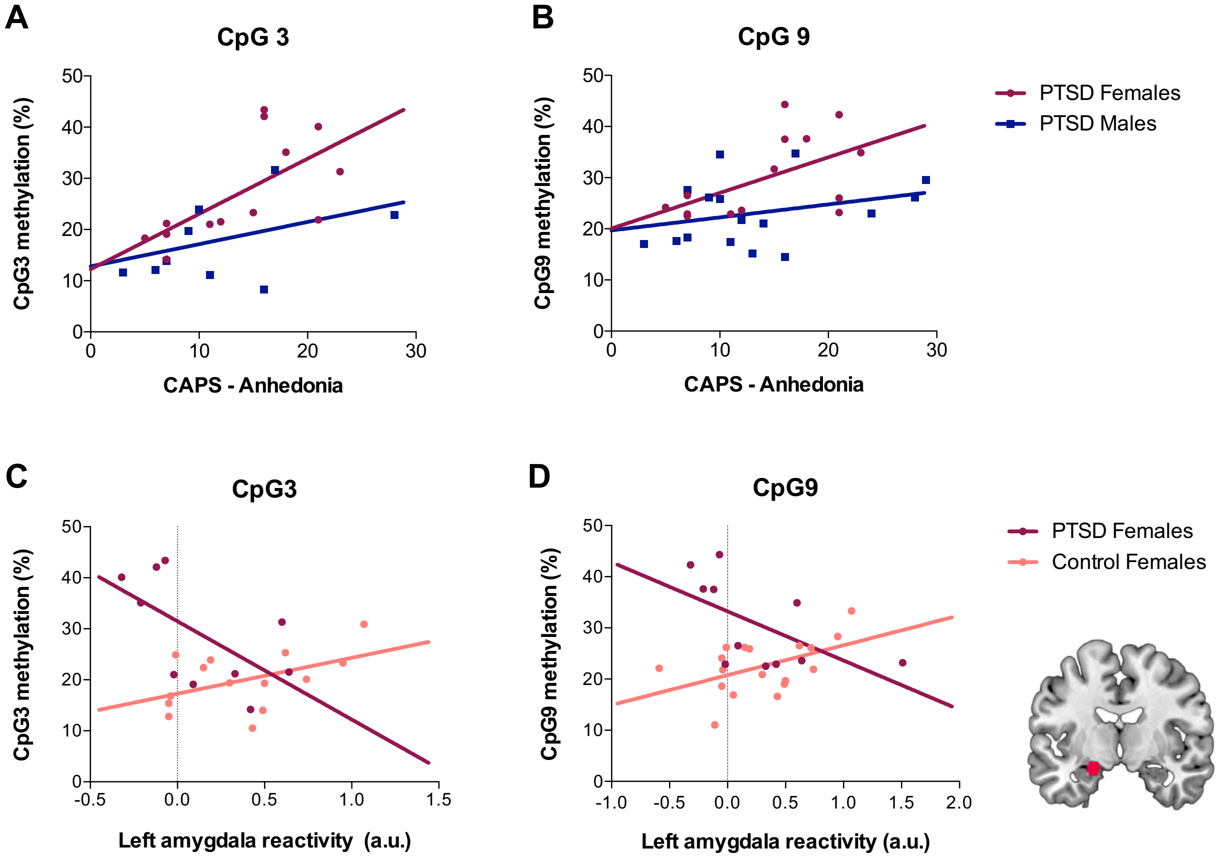

Fig. 3 Correlations between percentage *OXTR* methylation at CpG3 (A) and CpG9 (B) and CAPS subscale scores of anhedonia/emotional numbing symptom severity in male and female PTSD patients; Correlations between percentage *OXTR* methylation at CpG3 (C) and CpG9 (D) and left amygdala responses to negative faces in arbitrary units (a.u.) in female PTSD patients and female controls; Left amygdala sphere (peak-coordinates *xyz* −20, −8, 16) in which the associations were observed (insert bottom right).

a mixed-gender sample (Smearman et al., 2016). In a recent review of OXTR methylation studies, Maud et al. (2018) suggest that mixed findings may relate to psychiatric dimensions underlying different affective and anxiety disorders (e.g. (in)sensitivity for social cues, see also Puglia et al., 2018) or depend on specific OXTR SNPs allele frequencies (see Chagnon et al., 2015; Reiner et al., 2015). Also, though high genetic correlation between psychiatric disorders suggests commonality in the underlying pathogenic processes (Anttila et al., 2018), the contribution of single genes on these complex polygenic disorders will be small and may differ between disorders. Thus, heterogeneity in patient samples, and associations with specific symptom-clusters (see below) may explain part of the mixed results in psychiatric populations. Furthermore, heterogeneity between studies in (psychiatric) populations, methodological differences and small sample sizes further hamper reproducibility.

Female PTSD patients showed significant hypermethylation relative to control females and PTSD males, fitting with previously reported female-specific associations between OXTR methylation and psychopathology, early life adversity and socio-affective functioning (Gouin et al., 2017; Rubin et al., 2016). Several factors may explain this finding. Uddin et al. suggested that sex differences in DNA methylation during brain development, for example in estrogen receptor genes known to interact with OXTR, and sex-specific effects of stress on DNA methylation set the stage for future differential responses to adverse events and thereby the risk of PTSD development (Uddin et al., 2013). In line with this, Gouin et al. (2017) reported that females may be more sensitive to the impact of childhood adversity on OXTR methylation than males, and that OXTR methylation mediated the relation between childhood adversity and anxiousness. Thus, a combination of sex-differences in DNA methylation during development, sex-differences in effects of stress on DNA methylation, may lead to increased sensitivity to the impact of adversity on *OXTR* methylation and/or PTSD development.

Within PTSD females, we further observed that *OXTR* methylation was positively associated with clinician-rated anhedonia severity, which includes symptoms as social withdrawal, reduced positive affect and reduced interest in activities. Importantly, these findings were not driven by participants with comorbid MDD, nor by sex differences in anhedonia severity. Also, *OXTR* methylation was associated with lower amygdala reactivity to negative faces relative to neutral and positive faces, echoing previous associations between amygdala reactivity and *OXTR* methylation in SAD patients and healthy participants (Puglia et al., 2015; Ziegler et al., 2015). These effects were however no longer significant after correction for multiple comparisons and should be interpreted with caution. Interestingly though, high *OXTR* methylation was previously associated with reduced emotional recognition and neural responses during socio-affective tasks in healthy males and females (Haas et al., 2016; Puglia et al., 2018), and in female but not male schizophrenic patients and controls (Rubin et al., 2016). Increased OXTR methylation, supposedly associated with reduced responsiveness to oxytocin, may thus lead to reduced sensitivity to social cues. Anhedonia in PTSD has been associated with reduced sensitivity to positive (social) stimuli, particularly in females (Nawijn et al., 2015). In context of previous literature, the current findings could suggest that *OXTR* methylation may play a specific role in socio-affective deficits and anhedonia symptoms in PTSD, though replication is needed. Domain-specific associations may also explain inconsistencies in previous psychiatric studies, as the extent of socio-affective deficits may differ between disorders and within disorders between individual patients.

The currently observed sex-specific findings may further elucidate the biological mechanisms underlying sex differences in PTSD. However, replication is warranted and more fundamental research is needed. Considering the neurobiological and genetic complexity of psychiatric disorders, it is unlikely that one gene can explain large amounts of variance in psychiatric diagnoses. Our multilevel findings corroborate the notion that it is possible that gene-sets may affect specific biological mechanisms and symptom-clusters, and that different neurobiological mechanisms may underlie specific PTSD symptoms.

Some important limitations must be mentioned. First of all, the small sample size limits statistical power. Yet, including a homogeneous patient group and well-matched trauma-exposed controls will have optimized the ability to detect PTSD-associated differences. Thorough phenotyping allowed us to control for potential confounders and to associate *OXTR* methylation with clinically-relevant PTSD symptom-clusters and neuroimaging measures. Although the associations between anhedonia severity, amygdala reactivity and *OXTR* methylation were no longer significant after stringent correction for multiple comparisons, the large effect sizes correspond with those observed in previous psychiatric and healthy samples (e.g. Gouin et al., 2017; Puglia et al., 2015; Rubin et al., 2016; Ziegler et al., 2015). Another limitation is the fact that we could not control for menstrual phase, which may have affected *OXTR* methylation in women. Furthermore, given the cross-sectional nature of our study we cannot draw any causal inferences. In the absence of (central) *OXTR* expression data we can only infer the potential effects of *OXTR* methylation on (central) oxytocin functioning. High *OXTR* methylation-levels have been linked to lower central and peripheral *OXTR* expression, and decreased endogenous plasma oxytocin levels (Dadds et al., 2013; Gregory et al., 2009; Kusui et al., 2001), suggesting decreased central and peripheral oxytocin functioning.

Summarizing, we observed higher *OXTR* DNA-methylation levels in PTSD females compared to trauma-exposed female controls, but no association with PTSD-status in males. Within PTSD females, *OXTR* methylation was positively associated specifically with anhedonia symptoms, and with amygdala reactivity towards emotional faces. Considering our small sample size, more research is needed to confirm the current findings, and caution is necessary for the interpretation of our results. However, these findings are the first to suggest that *OXTR* methylation may play a role in socio-affective deficits in female PTSD patients. Though replication is warranted, the current findings add to the notion that the oxytocin system is involved in PTSD, and that neurobiological mechanisms involved in PTSD may differ between males and females.

Contributors

All authors contributed to and have approved the final manuscript.

LN, IMK, MvZ, PH, MAMMM and MO designed the current study. MO, DJV, MVZ, SBJK, LN, JLF designed the larger neuroimaging study. LN and SBJK performed all data acquisition. SBJK performed imaging data analysis. IMK, ANM and PH set-up and performed OXTR methylation assays. LN and IMK performed OXTR data analyses. LN, IMK, PH and MvZ drafted the manuscript, and all other authors contributed to the reading and editing of the manuscript. All authors contributed to and have approved the final manuscript.

Conflict of interest

All authors report no conflict of interest.

Role of funding source

Funding for this study was provided by grants from ZonMW, the Netherlands Organization for Health Research and Development (grant no. 91210041) and the AMC research council (grant no. 110614). ZonMW and the AMC research council had no further role in study design; in the collection, analysis and interpretation of data; in the writing of the report; and in the decision to submit the paper for publication.

Acknowledgments

The authors wish to thank the participants for their contribution to this study, and Renee Hutter, Gre Westerveld, Martha Hoofwijk and colleagues of the PDC outpatient clinic for their assistance in participant recruitment and data collection.

Supplementary material

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2018.10.006.

References

Anttila, V., Bulik-Sullivan, B., Finucane, H.K., et al., 2018. Analysis of shared heritability in common disorders of the brain. Science 360, eaap8757. doi:10.1126/science.aap8757, (80-.).

Bell, A.F., Carter, C.S., Steer, C.D., Golding, J., Davis, J.M., Steffen, A.D., Rubin, L.H., Lillard, T.S., Gregory, S.P., Harris, J.C., Connelly, J.J., 2015. Interaction between oxytocin receptor DNA methylation and genotype is associated with risk of postpartum depression in women without depression in pregnancy. Front. Genet. 6, 243. doi:10.3389/fgene.2015.00243.

Brewin, C.R., Andrews, B., Valentine, J.D., 2000. Meta-analysis of risk factors for posttraumatic stress disorder in trauma-exposed adults. J. Consult. Clin. Psychol. 68, 748–766.

Cappi, C., Diniz, J.B., Requena, G.L., Lourenço, T., Lisboa, B.C.G., Batistuzzo, M.C., Marques, A.H., Hoexter, M.Q., Pereira, C.A., Miguel, E.C., Brentani, H., 2016. Epigenetic evidence for involvement of the oxytocin receptor gene in obsessive-compulsive disorder. BMC Neurosci. 17, 79. doi:10.1186/s12868-016-0313-4.

Chagnon, Y.C., Potvin, O., Hudon, C., Préville, M., 2015. DNA methylation and single nucleotide variants in the brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) genes are associated with anxiety/depression in older women. Front. Genet. 6, 230. doi:10.3389/fgene.2015.00230.

Dadds, M.R., Moul, C., Cauchi, A., Dobson-Stone, C., Hawes, D.J., Brennan, J., Ebstein, R.E., 2013. Methylation of the oxytocin receptor gene and oxytocin blood levels in the development of psychopathy. Dev. Psychopathol. 1–8. doi:10.1017/S0954579413000497.

Frijling, J.L., van Zuiden, M., Nawijn, L., Koch, S.B.J., Neumann, I.D., Veltman, D.J., Olff, M., 2015. Salivary oxytocin and vasopressin levels in police officers with and without post-traumatic stress disorder. J. Neuroendocrinol. 27, 743–751. doi:10.1111/jne.12300.

Gouin, J.P., Zhou, Q.Q., Booij, L., Boivin, M., Côté, S.M., Hébert, M., Ouellet-Morin, I., Szyf, M., Tremblay, R.E., Turecki, G., Vitaro, F., 2017. Associations among oxytocin receptor gene (OXTR) DNA methylation in adulthood, exposure to early life adversity, and childhood trajectories of anxiousness. Sci. Rep. 7, 7446. doi:10.1038/s41598-017-07950-x.

Gregory, S.G., Connelly, J.J., Towers, A.J., Johnson, J., Biscocho, D., Markunas, C.a, Lintas, C., Abramson, R.K., Wright, H.H., Ellis, P., Langford, C.F., Worley, G., Delong, G.R., Murphy, S.K., Cuccaro, M.L., Persico, A., Pericak-Vance, M.a., 2009. Genomic and epigenetic evidence for oxytocin receptor deficiency in autism. BMC Med. 7, 62. doi:10.1186/1741-7015-7-62.

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. Proc. Natl. Acad. Sci. 113, E3816–E3823. doi:10.1073/pnas.1602809113.

Kimmel, M., Clive, M., Gispen, F., Guintivano, J., Brown, T., Cox, O., Beckmann, M.W., Kornhuber, J., Fasching, P.A., Osborne, L.M., Binder, E., Payne, J.L., Kaminsky, Z., 2016. Oxytocin receptor DNA methylation in postpartum depression. Psychoneuroendocrinology 69, 150–160. doi:10.1016/j.psyneuen.2016.04.008.

Klengel, T., Pape, J., Binder, E.B., Mehta, D., 2014. The role of DNA methylation in stress-related psychiatric disorders. Neuropharmacology 80, 115–132. doi:10.1016/j.neuropharm.2014.01.013.

Koch, S.B.J., van Zuiden, M., Nawijn, L., Frijling, J.L., Veltman, D.J., Olff, M., 2016a. Intranasal oxytocin administration dampens amygdala reactivity towards emotional faces in male and female PTSD patients. Neuropsychopharmacology 41, 1495–1504. doi:10.1038/npp.2015.299.

Koch, S.B.J., van Zuiden, M., Nawijn, L., Frijling, J.L., Veltman, D.J., Olff, M., 2016b. Intranasal oxytocin normalizes amygdala functional connectivity in posttraumatic stress disorder. Neuropsychopharmacology 41, 2041–2051. doi:10.1038/npp.2016.1.

Koch, S.B.J., van Zuiden, M., Nawijn, L., Frijling, J.L., Veltman, D.J., Olff, M., 2014. Intranasal oxytocin as strategy for medication-enhanced psychotherapy of PTSD: salience processing and fear inhibition processes. Psychoneuroendocrinology 40, 242–256. doi:10.1016/j.psyneuen.2013.11.018.

Krzyzewska, I.M., Ensink, J.B.M., Nawijn, L., Mul, A.N., Koch, S.B.J., Venema, A., Shankar, V., Frijling, J.L., Veltman, D.J., Lindauer, R.J.L., Olff, M., Mannens, M.M.A., van Zuiden, M., Henneman, P., 2018. Genetic variant in CACNA1C is associated with PTSD in traumatized police officers. Eur. J. Hum. Genet. 26, 247–257. doi:10.1038/s41431-017-0059-1.

Kusui, C., Kimura, T., Ogita, K., Nakamura, H., Matsumura, Y., Koyama, M., Azuma, C., Murata, Y., 2001. DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene suppression. Biochem. Biophys. Res. Commun. 289, 681–686. doi:10.1006/bbrc.2001.6024.

Love, T.M., Enoch, M.-A., Hodgkinson, C.a, Peciña, M., Mickey, B., Koeppe, R.a, Stohler, C.S., Goldman, D., Zubieta, J.-K., 2012. Oxytocin gene polymorphisms influence human dopaminergic function in a sex-dependent manner. Biol. Psychiatry 72, 198–206. doi:10.1016/j.biopsych.2012.01.033.

Maud, C., Ryan, J., McIntosh, J.E., Olsson, C.A., 2018. The role of oxytocin receptor gene (OXTR) DNA methylation (DNAm) in human social and emotional functioning: a systematic narrative review. BMC Psychiatry 18, 154. doi:10.1186/s12888-018-1740-9.

Nawijn, L., van Zuiden, M., Frijling, J.L., Koch, S.B.J., Veltman, D.J., Olff, M., 2015. Reward functioning in PTSD: a systematic review exploring the mechanisms underlying anhedonia. Neurosci. Biobehav. Rev. 51C, 189–204. doi:10.1016/j.neubiorev.2015.01.019.

Nawijn, L., van Zuiden, M., Koch, S.B.J., Frijling, J.L., Veltman, D.J., Olff, M., 2016. Intranasal oxytocin increases neural responses to social reward in post-traumatic stress disorder. Soc. Cogn. Affect. Neurosci. doi:10.1093/scan/nsw123.

Olff, M., Langeland, W., Witteveen, A., Denys, D., 2010. A psychobiological rationale for oxytocin in the treatment of posttraumatic stress disorder. CNS Spectrums 15, 522–530.

Pineles, S.L., Arditte Hall, K.A., Rasmusson, A.M., 2017. Gender and PTSD: different pathways to a similar phenotype. Curr. Opin. Psychol. 14, 44–48. doi:10.1016/j.copsyc.2016.11.002.

Puglia, M.H., Connelly, J.J., Morris, J.P., 2018. Epigenetic regulation of the oxytocin receptor is associated with neural response during selective social attention. Transl. Psychiatry 8, 116. doi:10.1038/s41398-018-0159-x.

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. Proc. Natl. Acad. Sci. USA doi:10.1073/pnas.1422096112.

Reiner, I., Van IJzendoorn, M.H., Bakermans-Kranenburg, M.J., Bleich, S., Beutel, M., Frieling, H., 2015. Methylation of the oxytocin receptor gene in clinically depressed patients compared to controls: the role of OXTR rs53576 genotype. J. Psychiatr. Res. 65, 9–15. doi:10.1016/j.jpsychires.2015.03.012.

Rubin, L.H., Connelly, J.J., Reilly, J.L., Carter, C.S., Drogos, L.L., Pournajafi-Nazarloo, H., Ruocco, A.C., Keedy, S.K., Matthew, I., Tandon, N., Pearlson, G.D., Clementz, B.A., Tamminga, C.A., Gershon, E.S., Keshavan, M.S., Bishop, J.R., Sweeney, J.A., 2016. Sex and diagnosis specific associations between DNA methylation of the oxytocin receptor gene with emotion processing and temporal-limbic and prefrontal brain volumes in psychotic disorders. Biol. Psychiatry Cogn. Neurosci. Neuroimaging 1, 141–151. doi:10.1016/j.bpsc.2015.10.003.

Shamay-Tsoory, S.G., Abu-Akel, A., 2015. The social salience hypothesis of oxytocin. Biol. Psychiatry doi:10.1016/j.biopsych.2015.07.020.

Smearman, E.L., Almli, L.M., Conneely, K.N., Brody, G.H., Sales, J.M., Bradley, B., Ressler, K.J., Smith, A.K., 2016. Oxytocin receptor genetic and epigenetic variations: association with child abuse and adult psychiatric symptoms. Child Dev. 87, 122–134. doi:10.1111/cdev.12493.

Stein, M.B., Jang, K.L., Taylor, S., Vernon, P.A., Livesley, W.J., 2002. Genetic and environmental influences on trauma exposure and posttraumatic stress disorder symptoms: a twin study. Am. J. Psychiatry 159, 1675–1681. doi:10.1176/appi.ajp.159.10.1675.

Uddin, M., Sipahi, L., Li, J., Koenen, K.C., 2013. Sex differences in dna methylation may contribute to risk of ptsd and depression: a review of existing evidence. Depress. Anxiety 10, 1–10. doi:10.1002/da.22167.

Unternaehrer, E., Bolten, M., Nast, I., Staehli, S., Meyer, A.H., Dempster, E., Hellhammer, D.H., Lieb, R., Meinlschmidt, G., 2016. Maternal adversities during pregnancy and cord blood oxytocin receptor (OXTR) DNA methylation. Soc. Cogn. Affect. Neurosci. 11, 1460–1470. doi:10.1093/scan/nsw051.

Unternaehrer, E., Luers, P., Mill, J., Dempster, E., Meyer, aH., Staehli, S., Lieb, R., Hellhammer, D.H., Meinlschmidt, G., 2012. Dynamic changes in DNA methylation of stress-associated genes (OXTR, BDNF) after acute psychosocial stress. Transl. Psychiatry 2, e150. doi:10.1038/tp.2012.77.

Unternaehrer, E., Meyer, A.H., Burkhardt, S.C.A., Dempster, E., Staehli, S., Theill, N., Lieb, R., Meinlschmidt, G., 2015. Childhood maternal care is associated with DNA methylation of the genes for brain-derived neurotrophic factor (BDNF) and oxytocin receptor (OXTR) in peripheral blood cells in adult men and women. Stress 18, 451–461. doi:10.3109/10253890.2015.1038992.

Wolf, E.J., Mitchell, K.S., Koenen, K.C., Miller, M.W., 2014. Combat exposure severity as a moderator of genetic and environmental liability to post-traumatic stress disorder. Psychol. Med. 44, 1499–1509. doi:10.1017/S0033291713002286.

Ziegler, C., Dannlowski, U., Bräuer, D., Stevens, S., Laeger, I., Wittmann, H., Kugel, H., Dobel, C., Hurlemann, R., Reif, A., Lesch, K.-P., Heindel, W., Kirschbaum, C., Arolt, V., Gerlach, A.L., Hoyer, J., Deckert, J., Zwanzger, P., Domschke, K., 2015. Oxytocin receptor gene methylation-converging multilevel evidence for a role in social anxiety. Neuropsychopharmacology doi:10.1038/npp.2015.2.