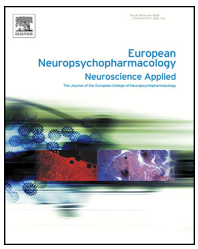
European Neuropsychopharmacology (2020) 39, 19–28



www.elsevier.com/locate/euroneuro

Methylation of oxytocin related genes and early life trauma together shape the N170 response to human faces

Franca H. Parianen Lesemann[a,*], Hannah Spencer[b,*], Estrella R. Montoya[a], Eline J. Kraaijenvanger[c], Yujie He[d], Susan Branje[e], Marco P. Boks[d], Peter A. Bos[f]

[a] *Department of Experimental Psychology, Utrecht University, Heidelberglaan 1, 3584 CS, Utrecht, the Netherlands*
[b] *Research Institute of Child Development and Education, University of Amsterdam, PO Box 15780, 1001 NG Amsterdam, the Netherlands*
[c] *Department of Child and Adolescent Psychiatry and Psychotherapy, Central Institute of Mental Health, Medical Faculty Mannheim / Heidelberg University, J5, D-68159 Mannheim, Germany*
[d] *Brain Center University Medical Center Utrecht, Utrecht, the Netherlands*
[e] *Department of Youth and Family, Utrecht University, Heidelberglaan 1, 3584 CS, Utrecht, the Netherlands*
[f] *Institute of Education and Child Studies, Leiden University, Leiden, the Netherlands*

Received 14 November 2019; received in revised form 24 June 2020; accepted 17 August 2020

KEYWORDS
Oxytocin;
Oxytocin receptor methylation;
Oxytocin structural gene methylation;
N170 EEG response;
Childhood trauma;
Sensitivity

Abstract
Childhood trauma fundamentally shapes social cognition and basic processing of social cues, which frequently cascade into adverse behavioral outcomes. Recent studies indicate that epigenetic changes in oxytocin functioning might contribute to these long-term effects, although a deeper understanding of the underlying mechanisms is still lacking. The electroencephalographic N170 response to faces might capture a neural response at the core of these interactive effects of oxytocin gene methylation and childhood adversity, given that this response is considered to reflect fundamental face processing, to be susceptible to oxytocin administration and also to be a biomarker of various psychiatric disorders. We assessed the N170 response to neutral faces in relation to participant's (81, women) recalled childhood trauma, methylation of their oxytocin structural (OXTg) and oxytocin receptor (OXTRg) genes, and endogenous lev-

* Corresponding author.
E-mail address: f.h.parianen-lesemann@uu.nl (F.H. Parianen Lesemann).

https://doi.org/10.1016/j.euroneuro.2020.08.008

els of cortisol and testosterone. Additionally, we investigated the interactive effect of OXTg methylation and CTQ across three face sets of varying maturity. Methylation of OXTg relates to a weakened N170 response towards adults, children and infants. Moreover, methylation of *both* OXTRg and OXTg shaped the directionality of adversity effects, predicting a weakened N170 response in those with high methylation and hyper-vigilance with participants with low methylation. Our results are the first to relate OXT(R)g methylation to the N170 response. They shed light on biological processes linking childhood adversity and epigenetic marks to altered behavior and potentially psychopathologies.

1. Introduction

Traumatic experiences in childhood fundamentally shape cognitive development (De Bellis et al., 2005, Oitzl et al., 2010), including the functioning of brain areas involved in anxiety and emotion regulation (Tottenham, 2012). Indeed, effects of early adversity can be found in the most basic aspects of stimulus perception and processing (Teicher et al., 2016). These fundamental alterations, might be at the basis of adverse behavioral effects associated with early adversity, such as anxiousness, insecure attachment, distrust, and excessive vigilance, as well as related psychopathologies (for a review see Brietzke, 2012). For example, traumatized children's over-attentiveness towards threat cues increases overall symptoms of anxiety (Shackman et al., 2007) and mediates future outcomes like the risk for PTSD (Fani et al., 2011). It is thus important to understand the biological mechanisms through which childhood adversity shapes fundamental social processing later in life. Yet, while the effects of trauma on social cognition and development are described in a multitude of studies, the underlying biological processes remain unclear. Recent research indicates that epigenetic changes altering oxytocin functioning might underlie some of the effects of adversity (Gouin et al., 2017, Milaniak et al., 2017, Simons et al., 2017). The current study adds to this research, by assessing how methylation of oxytocin structural gene (OXTg), or oxytocin receptor gene (OXTRg) and self-reported recall of childhood trauma shape participant's response to neutral faces.

Administration studies frequently showed how oxytocin affects behavioral and neural responses to ambiguous social stimuli, associating it with increased trust and emotion regulation as well as decreased anxiety and threat sensitivity (Bos et al., 2012). Variations in oxytocin receptor genotype were related to attachment anxiety (Costa et al., 2009), trust, and amygdala volume- a region involved in the detection of emotionally salient or potentially threatening stimuli (Nishina et al., 2018). However, results indicate strong individual variation in oxytocin functioning and its relation to social anxiety (Beery, 2015). Such variation might rely on epigenetic regulation through DNA methylation (Kraaijenvanger et al., 2019), i.e. the attachment of methyl groups to sequences of the DNA altering transcription of the targeted gene (Szyf and Bick, 2012), responding to environmental needs (Bird, 2007, Dupont et al., 2009, Maccari et al., 2014). DNA methylation impacts oxytocin functioning, by affecting production at the oxytocin gene (OXTg) or -receptor distribution and density at the oxytocin receptor gene (OXTRg) (Kusui et al., 2001, Perkeybile et al., 2018). In accordance methylation levels of OXTRg correlate with attachment anxiety (Ebner et al., 2018), attachment avoidance scores (Ein-Dor et al., 2018) and the risk for PTSD (Nawijn et al., 2018). On a neural level, OXTRg methylation predicted amygdala volume (Lancaster et al., 2018) and coupling with regions relevant for emotion regulation (Puglia et al., 2015). While less is known about the role of OXTg in social cognition, it negatively relates to secure attachment (Haas et al., 2016). Taken together, these findings indicate that individual differences in oxytocins's (epi)genetic predispositions may affect a range of social cognitive processes, potentially facilitating social anxiousness.

Important factors that shape the oxytocin system are early life attachment and adversity. For instance, early adversity seems to alter sensitivity to oxytocin administration effects (Bartz et al., 2010, Rockliff et al., 2011), also in tasks involving trust (Ebert et al., 2013). Rodent studies associate these long-term effects with OXTRg methylation and a resulting decrease of oxytocin receptors in the brain (Perkeybile et al., 2018), as well as with altered behavioral outcomes and increased responsivity to a stressor (Beery et al., 2016). In humans, there are obvious limitations to establishing causal links between adversity and OXT(R)g, resulting in variable findings on this relation, even following severe adversity (for a review see Kraaijenvanger, 2019). Yet, several studies found *interactive* effects, indicating that OXTRg methylation moderates, or even mediates, the effects of adversity on childhood anxiousness (Gouin et al., 2017) and adult feelings of distrust (Simons et al., 2017), depression and anxiety (Smearman et al., 2016), or effects of prenatal stress on conduct disorder (Milaniak et al., 2017). Furthermore, in samples with clinical disorders like schizophrenia, depression or anxiety disorders – all of which are characterized by alterations in basic social processing and anxiousness - childhood trauma (Carr et al., 2013, Martins et al., 2011) often seems to go along with alterations in oxytocin expression and sensitivity or in OXTRg methylation (Chatzittofis et al., 2014, Kraaijenvanger et al., 2019, Meinlschmidt and Heim, 2007). These epigenetic predispositions in turn predict decreased abilities for emotion recognition (Rubin et al., 2016) and regulation (Hoge et al., 2008, Laeger et al., 2012, Schmidt et al., 2010). Together, these findings suggest that OXT(R)g methylation might constitute a biological factor underlying long-term effects of early adversity on social processing. Coherently, we want to assess interactive effects of OXT(R)g methylation and early adversity on neural and behavioral responses towards faces of adult strangers.

As outcome measure, we focus on the N170 response, an early and robust neural component in face processing (Hinojosa et al., 2015) that increases with attentiveness and salience (Churches et al., 2010, Hinojosa et al., 2015,

Noll et al., 2012), but also perceived threat or emotionality (Montalan et al., 2008, Ratner and Amodio, 2013). This response was altered after oxytocin administration (Peltola et al., 2018) and in a number of disorders associated with OXT(R)g methylation, including social anxiety (Feuerriegel et al., 2015). Hence, it might capture a neural response that underlies the relay from childhood adversity and respective epigenetic effects to altered behavior and psychopathologies. Yet, no study to date assessed a link between childhood trauma, OXT(R)g methylation levels and the N170 response. By probing this link in a healthy, neuro-typical group of participants, we hope to shed light on a potential population general effect at the basis of the observed manifold alterations. We will then investigate whether the effect depends on face category by looking into the same participant's responses towards non-threatening, i.e. neutral infant and children, faces. Finally, we control for basal endocrine values of testosterone and cortisol, to mitigate their potential impact given testosterone's role in trust perception (Bos et al., 2012) and cortisol's interaction with childhood trauma and the oxytocin system (Heinrichs et al., 2003, McGowan et al., 2009). We expect to observe a negative relationship between the amplitude of participant's N170 response, their self-reported recall of childhood trauma and their degree of OXT(R)g methylation.

2. Methods and materials

2.1. Participants

81 healthy women (*M* age =23.60, *SD* = 0.44) participated, most of which were Caucasian Dutch (*n*=2 with dual nationality), non smokers (*n*=51, 1 per month or less: $n = 8$, smoke daily or weekly $n = 19$, n.a.:$n = 3$), and of higher professional or University education ($n = 62$; primary or secondary vocational education: $n = 16$). All women were part of the RADAR (Research on Adolescent Development And Relationships) study and screened through telephone interviews for a history of endocrinal, neurological, or psychiatric conditions, and use of related medication. Since the study was involved in a bigger project assessing caregiving behaviors prior to motherhood, only nulliparous women were approached.

2.2. Experimental procedure

Participants were asked to abstain from cigarettes, medication and/or recreational drugs, food and drinks, other than water, for one hour before the experiment. Upon arrival, they were informed about task procedures and gave written informed consent. After task completion, participants filled out the Dutch version of the Childhood Trauma Questionnaire (CTQ). They were then debriefed and compensated.

The study was conducted in accordance with the latest version of the Declaration of Helsinki and was approved by the medical ethical committee of the University Medical Center Utrecht.

2.3. Stimuli and behavioral ratings

Stimuli for the trust task were taken from the Oslo Face Database and consisted of 15 male and 15 female faces, presented in random order, followed by 1 s of blank screen. After each face, participants reported perceived trustworthiness (1 - *not trustworthy at all*, 9 - *very trustworthy*). Inter-trial durations varied according to reaction times.

2.4. Assessment of self-recalled childhood trauma

Recall of childhood trauma was assessed after the trust task using the short version of the CTQ (Bernstein et al., 2003). Total score of all items was included in the analysis (*M*=32, *SD*=7.73, range=24-61).

2.5. EEG measurements

We recorded EEG data using the Biosemi ActiveTwo system (Biosemi, Amsterdam, The Netherlands), through 32 AG/AgCl pin electrodes, placed according to the International 10/20 electrode placement standard at 2048 Hz sampling rate. As the ground reference point, we placed passive driven right leg (DRL) electrode and active common mode sense (CMS) on central sagittal midline scalp. Horizontal and vertical eye movements were measured through electrodes placed besides, above and below the left eye as well as besides the outer canthus of both eyes.

We down-sampled raw EEG traces to 256 Hz and re-referenced them to the average activity across all electrodes. Data were 0.1-30 Hz band pass filtered, with a 24 dB roll-off per octave and a notch filter of 50 Hz, segmented (200 ms pre-stimulus - 1000 ms post-stimulus) and baseline corrected relative to the 200 ms pre-stimulus interval. Segments containing eye movements and blinks were corrected using the Gratton & Coles method (Gratton et al., 1983). Artifacts were rejected through semi-automatic inspection of EEG channels, with maximal allowed voltage steps of 50 μV, maximal allowed amplitude differences of 100 μV within 200 ms segments and lowest allowed activity of 0.5 μV in 200 ms intervals. Channels, with isolated artifacts in $>$ 20% of trials (in 7 participants) were removed from analysis. Data sets of twelve participants were excluded due to excessive artifacts ($\geq$ 25% of trials). On average, artifacts were detected in 8.16% of all trials.

Statistical analyses were performed for data recorded from the P7 and P8. Selection of time windows and electrodes was based on previous research (Endendijk et al., 2018). Specifically, N170 was quantified post-stimulus from 162-212 ms, whereas a more negative signal can be interpreted as a stronger response.

2.6. OXTRg and OXT(R)g methylation assessment

After task execution, a salivary DNA sample was collected for the methylation assessment using Oragene DISCOVER (OGR-500) DNA collection kits (DNA Genotek Inc., Ottowa, Canada). After DNA extraction, DNA methylation was quantified for the *OXT* gene (GRCh38/hg38, chr20: 3071297-3071697) and the *OXTR* gene (GRCh38/hg38, chr3: 8769043-8769159 (containing CpG site -934 (hg38, 3:8769121)) relative to full and absent methylation using methylation sensitive high resolution melting curve analysis (MS-HRM) (Wojdacz and Dobrovic, 2007). For details, see supplementary material.

2.7. Basal hormone assessments

Hormone analysis of cortisol and testosterone levels was performed using SaliCaps. Saliva samples were collected upon arrival and stored at -20°C until analysis. Laboratory analyses were performed at the Department of Biopsychology, TU Dresden, Germany, where

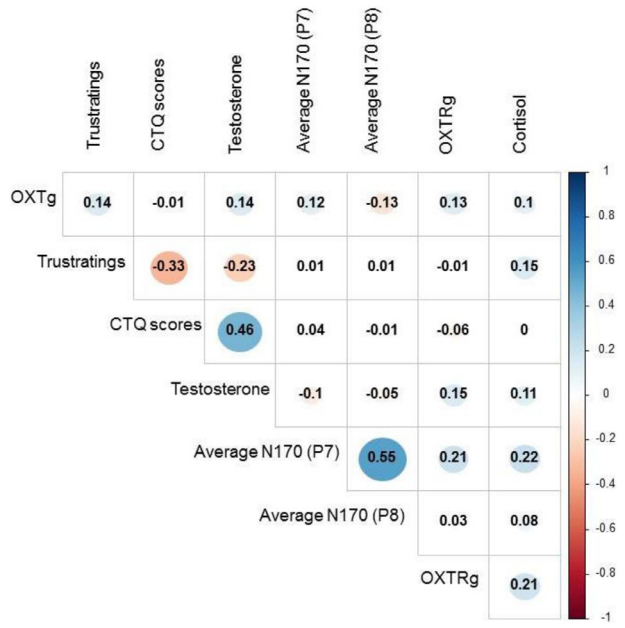


Fig. 1 Correlation Matrix of independent variabels.

samples were centrifuged at 3000 rpm for 3min and hormone concentrations were measured using liquid chromatography-tandem mass spectrometry (LC–MS). The mean inter-assay coefficients of variations was 4.54% ($SD = 0.03$).

2.8. Statistical analyses

To avoid interdependence, all effects were assessed in separate models including either OXTg or OXTRg methylation.

First, OXTg methylation and OXTRg methylation were regressed on CTQ scores (for correlations see Fig. 1). We then calculated, two linear regression, assessing (interactive) effects of CTQ scores, and OXT(R)g methylation on participant's average N170 as measured by P7 and P8 electrodes (Base models, Fig. 2). Since no effects were found on P8, it was dropped from analysis.

Second, we amended this model through endogenous levels of testosterone and cortisol and their potential interactions with CTQ scores and OXT(R)g methylation. Endocrine and methylation measures were scaled since they do not contain a meaningful value of zero.

Third, we probed the effect's reliability and relation to image characteristics through a mixed model analysis, including scaled and averaged trust-ratings of the pictures (hereafter, trustworthiness). Subject and image were added as random effects resulting in maximal model structure (Barr et al., 2013). The same model structure was used to assess participant's behavioral responses. Responses given in less than 50 ms were excluded from analyses, to avoid arbitrariness.

$N170 \sim trustworthiness_{scaled} * OXT(R)g_{scaled} * CTQ\_Total * (testosterone_{scaled} + cortisol_{scaled}) * + (1|Subject) + (1|Image)$

At last, we probed effects of the linear regressions in three more existing datasets. These datasets were collected in the context of other studies from our lab and consisted of the same participants' response to 27 infant faces (2/ 3 manipulated to appear high, respectively low in cuteness) and 80 children's faces measured across two tasks, during which participants manipulated viewing time (Reward Task), or rated 'subjective liking'. Regressions were similar with the addition of image characteristics manipulated in those tasks (cuteness, group membership).

Table 1 Participant's CTQ scores by subscales categorized according to CTQ manual (Bernstein and Fink, 1998).

	None	Low	Moderate	Severe
Emotional abuse	59	16	5	1
Physical abuse	77	2	2	0
Sexual abuse	76	3	2	0
Emotional neglect	53	19	8	1
Physical neglect	61	15	5	0

All analyses were carried out in R (Version 3.4.1.) (Team, R. 2013) using the checkpoint function. Type III ANOVAs using the lmer and lm4 packages (Kuznetsova et al., 2014) were used to conduct significance omnibus tests. Post Hoc contrasts were computed with the "lsmeans" package (Lenth, 2016). We report 95% confidence intervals and p-values.

3. Results

3.1. Descriptive statistics

A number of participants (n=74) reported at least some form of adverse experiences (see Table 1). Additionally, 8 participants reported moderate to severe physical or emotional abuse and 14 participants reported moderate to severe physical or emotional neglect (D. (Bernstein and Fink, 1998)). 5 participants reported some form of sexual abuse, which is considered particularly severe (Glaesmer et al., 2013).

3.2. Methylation and CTQ

As expected, given the relatively low prevalence of severe trauma in our sample, CTQ scores were not directly related to OXTg methylation ($F_{(1|79)}$=0, p=0.9820) or OXTRg methylation ($F_{(1|79)}$=0.3425, p=0.56). We thus continue with the moderation analysis.

3.3. Methylation and endocrine measures

CTQ scores were not related to cortisol levels (p=0.92) but to increases in testosterone (M=0.3, SE=0.07, $F_{(1|79)}$=21.18, p<0.0001). Variance inflation factors, to test for the risk of multi-collinearity, indicated a value of 1.268 (below the common threshold of 4). Yet, to be precautious, we began our analysis with a smaller model, not including endocrine measures.

3.4. Trust task

3.4.1. EEG measurements, N170 response

Base model

P7.

OXTg. The model revealed a main effect of OXTg methylation ($F_{(1|76)}$=4.8, p=0.03145) and an interaction between OXTg methylation and CTQ scores ($F_{(1|76)}$=5.81,

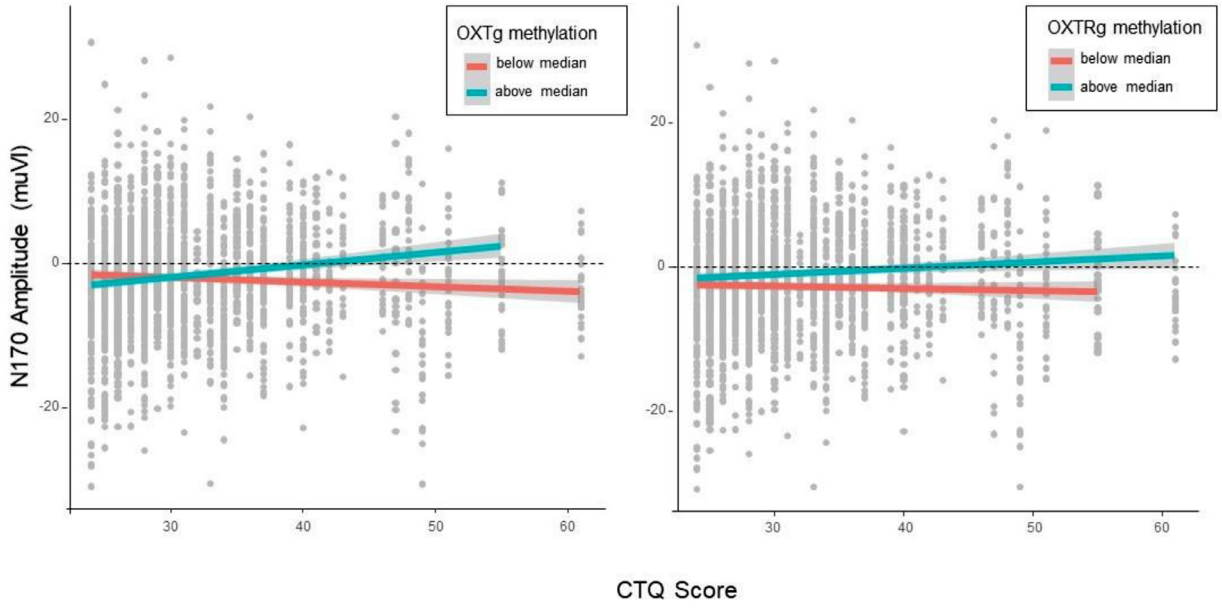


Fig. 2 Effect of CTQ score on the N170 component (negativity indicating a stronger response), plotted by median split of OXTg (left panel) and OXTRg (right panel) methylation levels. Blue lines indicating high methylation levels. A median split was conducted for visualization purposes only. OXTg=Oxytocin gene; OXTRg=Oxytocin receptor gene, CTQ_Total=Sum answers childhood trauma questionnaire.

p=0.01836), whereas only the interaction would remain significant after Bonferroni correction (p<0.025).

Post Hoc tests showed differential effects of CTQ scores depending on OXTg methylation (slope difference ±1 *SD*, *M*=-0.37, *SE*=0.15, $t(76)$=-2.41, p=0.0184), with early adversity decreasing N170 intensity in participants with high to medium degrees of methylation (mean+1*SD*: *M*=0.26, *SE*=0.11, 95 % CL [0.03, 0.48], $t(76)$=2.273, p=0.0258; mean: $t(76)$=1.207, p=0.2313, mean-1*SD* $t(76)$-1.445=0.1527).

OXTRg. This model showed no effects of OXTRg methylation (p=0.86) or CTQ scores (p=0.65).

P8.

The P8 was not affected by either methylation levels (OXTg: p=0.15, OXTRg: p=0.4964) or CTQ scores (OXTg model: p=0.7, OXTRg model: p=0.94).

All subsequent analyses are thus conducted on the P7.

3.4.2. Combinatory model with endocrine measures

OXTg: Linear models confirmed the main effect of OXTg methylation ($F_{(1|68)}$=7.2689, p=0.008835) and the interaction between OXTg and CTQ ($F_{(1|68)}$=8.0897, p=0.005876). In addition, this model indicated a main effect of CTQ score ($F_{(1|68)}$=5.7138, p=0.019608).

Post hoc tests confirmed differential effects of CTQ scores depending on OXTg methylation (slope difference ±1 *SD*, *M*=-0.58, *SE*=0.2, $t(68)$=-2.869, p=0.0055), with CTQ scores weakening the N170 response especially in participant's with high methylation (mean+ 1*SD*, *M*=0.17, *SE*=0.07, 95 % CL [0.03, 0.31], $t(68)$=2.396, p=0.0194; mean: *M*=0.46, *SE*=0.14, 95 % CL [0.18, 0.74], $t(68)$=3.270, p=0.0017, mean -1*SD*, p=0.2452).

OXTRg: When taking into account hormonal variation, the model revealed an interaction between OXTRg methylation and CTQ score ($F_{(1|68)}$=4.1520, p=0.04548), although this would not remain significant after Bonferroni correction (<0.025).

Post Hoc tests indicated differential effects of CTQ score depending on the level of OXTRg methylation (slope difference ±1*SD*, *M*=-0.45, *SE*=0.22, $t(68)$=-2.024, p=0.0469), with CTQ scores decreasing intensity of the N170 component mainly in participants with high OXTRg methylation (mean+ 1*SD*, *M*=0.31, *SE*=0.13, 95 % CL [0.04, 0.57], $t(68)$=2.278, p=0.0259; mean: *M*=0.08, *SE*=0.07, 95 % CL [-0.06, 0.22], $t(68)$=1.199, p=0.2348).

3.4.3. Additional mixed model analysis

OXTg: The model confirmed main effects of CTQ score ($F_{(1|67.19)}$=5.6696, p=0.020102) and OXTg methylation ($F_{(1|67.58)}$=7.1611, p=0.009339), and the interaction between OXTg methylation and CTQ score ($F_{(1|67.64)}$=8.0056, p=0.006123). There were no interactions with image trustworthiness.

OXTRg: The model confirmed the interaction between OXTRg methylation and CTQ score ($F_{(1|67.50)}$=4.3325, p=0.04119), although this would not remain significant after Bonferroni correction.

3.5. Behavior

OXTg: Linear models revealed a main effect of image trustworthiness ($F_{(1|\ 2327.03)}$=14.0075, p=0.0001865), and CTQ ($F_{(1|68.99)}$=4.7916, p=0.0319847). testosterone interacted with trustworthiness ($F_{(1|2327.03)}$=9.2264, p=0.0024120). Three-way interactions were found between testosterone, CTQ score and trustworthiness ($F_{(1|2327.03)}$=13.1111, p=0.0002998), and between testosterone, CTQ score and OXTg methylation ($F_{(1|2327.03)}$=6.5084, p=0.0108004). A four way interaction occurred between testosterone,

OXTg methylation, CTQ score and trustworthiness ($F_{(1|2372.02)}$=7.6598, p=0.0056910). All but the main effect of CTQ remain significant after Bonferroni correction (p<0.025).

Post hoc tests for interactions between CTQ score, testosterone and trustworthiness show that the slope relating image trustworthiness and participants' ratings, i.e. their sensitivity for trust cues, flattens with increasing testosterone levels predominantly in participants recalling high CTQ scores (slope-difference at T ±1*SD* in participants with CTQ+1 *SD*, es=0.29, *SE*=0.08, t(2327.03)=3.514, p=0.0005; T±1*SD* mean CTQ: es=0.08, *SE*=0.07, t(2372.03)=1.091, p=0.2754).

While we tested for three- and four-way interactions between endocrine and methylation measures, we did not further interpret the results due to a lack of statistical power of the dataset (and unclarity of the underlying biological mechanisms). Nevertheless, a summary is provided in the Supplementary materials.

OXTRg: The mixed model confirmed main effects of trustworthiness ($F_{(1|2327)}$=18.1671, p<0.0001) and CTQ score ($F_{(1|68.97)}$=8.4906, p=0.004809), as well as the interaction between trustworthiness, testosterone and CTQ scores ($F_{(1|2327.05)}$=6.5014, p=0.010843), which followed a similar pattern as described above (see Supplementaries).

3.6. Validation across different face sets

(for detailed analysis and figure see supplementary materials)

We validated the main effect of OXTg methylation for infant faces ($F_{(1|57)}$=21.1892, p<0.0001) and the two tasks involving children's faces (Reward task: $F_{(1|69)}$=5.5197, p=0.02167; Liking task: $F_{(1|69)}$=5.4516, p=0.02247). Also the interaction between OXTg methylation and CTQ score was replicated towards infants ($F_{(1|57)}$=20.4212, p<0.0001) and children's faces (Reward task: $F_{(1|69)}$=5.59195, p=0.01757; Liking task: $F_{(1|69)}$=5.1003, p=0.02709). There was no interaction with other image characteristics (cuteness, group membership).

4. Discussion

The current study examined whether childhood adversity interacts with OXT(R)g methylation in affecting early social processing. Our results show that OXTg methylation is associated with left hemispheric N170 activation towards adult faces, and that the impact of early adversity is modified by OXTg as well as OXTRg methylation levels, although the effects of OXTRg were weaker and did not always survive Bonferroni correction. Notably, the effects of OXTg methylation and its interaction with CTQ were sustained in mixed model analysis, and when taking into account potential influences of endocrine measures. Moreover, we validated this (interactive) effect in three separate tasks assessing participants' response to children's and infants' faces. By comparison, OXTRg effects were found in linear regression as well as mixed model analysis but only if endocrine variables were taken into account. An important role of endocrine variables was also indicated by testosterone's effect on behavioral measures of sensitivity to trustworthiness and its interaction with CTQ and OXTg. Meanwhile, there was no main effect of OXT(R)g methylation on behavioral ratings.

In our data OXTg methylation was associated with an increased N170 response towards all face sets analyzed, irrespective of trustworthiness, and – as indicated by the replication analysis - their maturity, cuteness or group membership. These results showed for the first time that OXTg methylation is related to a more pronounced fundamental processing component, which reflects salience and attention (Churches et al., 2010, Hinojosa et al., 2015, Noll et al., 2012) and which is considered a biomarker for a number of socio-emotional psychopathologies (Feuerriegel et al., 2015). This effect might underlie the negative relationship between OXTg methylation, emotion recognition and secure attachment (Haas et al., 2016). Even more so, it might be at the basis of associations between OXT(R)g methylation and disorders associated with alterations of the N170 response, including schizophrenia, anxiety disorders, and autism spectrum disorders (Brietzke et al., 2012; Carr et al., 2013, Kraaijenvanger et al., 2019). Thus, our results are consistent with the presence of a biological mechanism that links early experience and epigenetic marks to individual differences in social processing and related psychopathologies. As expected, CTQ scores did not directly affect OXT(R)g methylation in this neuro-typical participant group. Yet, even in this population, we consistently find those variables to interact, emphasizing the potential relevance of their association in shaping social cognition (Gouin et al., 2017, Simons et al., 2017, Smearman et al., 2016). Indeed, while most methylation studies in humans, as ours, remain correlational (Kraaijenvanger et al., 2019), and a longitudinal measurement of OXTRg methylation would be necessary to confirm changes over time, rodent studies suggest a causal link between early adversity, alterations of the oxytocin system and OXTRg methylation levels (Beery et al., 2016, Champagne, 2011, Perkeybile et al., 2018). Moreover, in humans, childhood adversity altered the responsiveness to administered oxytocin (Bartz et al., 2010, Ebert et al., 2013, Huffmeijer et al., 2013, Rockliff et al., 2011). Additionally, OXTRg methylation moderated effects of childhood adversity on girl's anxiousness (Gouin et al., 2017) and adult's feelings of distrust and anxiousness, in depression and other psychopathologies (Simons et al., 2017, Smearman et al., 2016). Our findings now show that this interaction might affect social cognition on a level as early and basic as the N170 response.

Particularly, an undifferentiated increase of N170 response as indicated by the main effect of OXTg methylation is considered an indicator of hyper-vigilance, which can be found in social phobia and social anxiousness (Kolassa and Miltner, 2006), whereas this response decreased after cognitive behavioral therapy (Cao et al., 2017). Our findings might thus suggest a role of OXTg methylation in anxiousness. An important aspect for interpretation might be the effect's lateralization, since the underlying mechanism is still under debate. While several studies found right-lateralized N170 responses to faces (Feuerriegel et al., 2015) this pattern seems to differ for instance in women (Proverbio et al., 2010), or in the context of psychiatric diseases (Feuerriegel et al., 2015).

Beyond that, our results show that the directionality of N170 effects is in fact shaped by the interaction of OXT(R)g methylation and early adversity. That is to say, early adversity either de- or increased the N170 component depending on the level of methylation. Given the relevance of adversity in affecting cognition and fostering the development of psychopathologies (Teicher et al., 2016), predictions from this interaction might actually be most relevant for our understanding of OXT(R)g methylation effects on social cognition.

On the one hand, CTQ scores in relation to high degrees of OXTg or OXTRg methylation predicted an attenuated N170 response (OXTg: p=0.0194,OXTRg: p=0.0259). In contrast to hyper-vigilance, this weakened response might reflect difficulties in perceiving and processing socially salient information, particularly regarding facial features, which is seen for example in prosopagnosia, but also in schizophrenia and sometimes in autism spectrum disorder (Feuerriegel et al., 2015). Our study is the first to relate this decrease in N170 activation to methylation of OXT(R)g. Again, this basic mechanism might inform previous findings on OXTRg methylation and social perception. Particularly, Puglia and colleagues have found OXTRg methylation to predict decreased coupling and over-activation in attentional (and emotional-) control related networks (Puglia et al., 2018; Puglia et al., 2015) which they interpreted as compensation mechanism for decreased face processing abilities, building on observations in autism (Herrington et al., 2015) and a correlation of their findings with autistic traits (Puglia et al., 2018). Our study now shows that OXT(R)g methylation might indeed hamper a core component related to structural face processing. Future research should investigate this link in clinical populations and bridge thus far unrelated strains of research, which associate autism and schizophrenia to attenuated N170 response or OXTRg hypermethylation (Feuerriegel et al., 2015, Maud et al., 2018). As a biological mechanism, OXTRg hypermethylation, might lower oxytocin receptor expression and sensitivity (Kusui et al., 2001, Perkeybile et al., 2018), which could interfere with its role in detecting socially salient information or processing of the eye region and top-down regulation of stimuli responses (Bos et al., 2012). OXTg methylation might exert a similar impact by limiting the overall degree of oxytocin, albeit its biological effects are yet to be investigated.

On the other hand, in participants self-reporting high degrees of adversity, *low* methylation of both OXTRg and OXTg went along with a hyper-vigilant N170 response, akin to observations in social anxiety and social phobia (Feuerriegel et al., 2015). This positive relation might underlie previous findings, relating OXTRg hypomethylation to social anxiety and phobia (Maud et al., 2018). Taken together, these findings might suggest that early adversity is linked to adult anxiety through hypomethylation. On a physiological level, OXTRg hypomethylation might result in hypersensitivity to oxytocin and thus an excessive response to socially salient information (Maud et al., 2018). Indeed, a recent study associated low OXTRg methylation with increased activation of the visual cortex during social interaction (Chen et al., 2019).

Notably, the association of low methylation and hypervigilance in participants with high CTQ scores stands in contrast to the directionality of the main effect, indicating an stronger N170 response with OXTg methylation. Coherently, a number of studies found OXTRg methylation effects to depended on individual factors like trait anxiety, autistic traits (Puglia et al., 2018) and gender (Schneider-Hassloff et al., 2016). Our results now shows that the effect of OXT(R)g methylation might also depend on early experience. Arguably, these differential findings might reflect a common biological mechanism. For instance, levels of plasma oxytocin were shown to differ in populations with anxiety, autism spectrum disorders or experienced childhood trauma (Al-Ayadhi, 2005, Heim et al., 2009, Tops et al., 2007), and the effect of OXTRg methylation was shown to differ depending on plasma oxytocin levels (Ebner et al., 2018, Ziegler et al., 2015). While it is yet unclear to what extent peripheral measures of oxytocin reflect oxytocin levels in the brain, interactions between oxytocin fluctuation and methylation, might be an interesting target for future research. The same holds true for the role of genotypes or gender, which we could not assess with our relatively small and exclusively female sample. On a more general note, other factors such as age, smoking, ethnicity or medication use might affect methylation (Kader and Ghai, 2017) and future research in a bigger and more variable samples will have to show, how such individual differences might have influenced our results. Moreover, biological research might soon reveal more information on how tissue type, cell type composition and sample cell origin may have affected our results. In fact, one limitation of our study is that we did not controll for cell type extracted from the saliva samples. Yet, the notion that we reliably and consistently find our effect across a number of measures, even using an indirect, salivary, measure of methylation, and a relatively small sample of participants, underscores its potential strength.

Lastly, we found effects of OXTg methylation, but not OXTRg methylation, to generalize towards infant faces. This is in line with some previous studies, which have found the N170 response – and the effect of oxytocin on it – to be independent of face maturity (Peltola et al., 2018, Spencer et al., 2018). Similarly, a recent study found OXTg expression across brain tissue to be less specific to social cognition than that of OXTRg (Rokicki et al., 2019), indicate a more general effect of OXTg methylation. It would thus be interesting to assess its effect on the N170 towards non-social stimuli, or on other EEG components related to facial processing such as P1 and P2. Either way, this OXTg effect (and its interaction with CTQ) might be particularly *consequential* in response to children – given the importance of sensitivity in parenting and oxytocin's role in it. Indeed, early adversity might alter oxytocin effects on responsivity to children (Bakermans-Kranenburg et al., 2012, Riem et al., 2017). Moreover, our results indicate that impacts of childhood adversity could be bi-directional, resulting in reduced processing or over-sensitivity and hyper-vigilance. This variability could underlie previously inconsistent results regarding the relation between N170 and maternal behaviors, and its association with the N170 parenthood in general (Proverbio et al., 2006), as well as mother's depressive symptoms (Noll et al., 2012) or insecurity (Fraedrich et al., 2010). Even more so, potential hyper-vigilance should be considered when assessing the risks and benefits of administering

oxytocin to treat social anxiety or postpartum depression symptoms.

Regarding the hormonal effects, testosterone negatively predicted sensitivity to faces' trustworthiness, especially in participants who experienced early adversity. These findings add to a number of studies emphasizing testosterone's importance in trust (Bos et al., 2012) and imply a role of early adversity in this interaction. However, this effect was not reflected in the N170 response. Coherently, OXT(R)g methylation affected the N170 but not trust ratings, indicating that these measures might have been relatively independent. At last, we find a potential interaction between OXTg methylation and testosterone levels, which is difficult to interpret, given our limited knowledge of underlying biological mechanisms. However, it is in line with other studies emphasizing the relevance of assessing epigenetic predispositions when studying endocrine responses (Chen et al., 2019).

In sum, our study points towards an interaction of early adversity and epigenetic differences of the oxytocin system, which together shape a basic component of facial processing. Such a mechanism might contribute to the etiology of the diverse types of pathologies associated with social abnormalities. The resulting findings give valuable insights for further studies of the N170 response as well as the oxytocin system, by identifying a source of individual variation, as well as a potential biomarker for interventions.

Role of the funding source

The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

Contributors

All authors made a significant intellectual contribution to the study. Moreover, the work in this paper was supported by a grant from the Netherlands Society of Scientific Research to P.A.B. (451-14-015). In addition, Data of the RADAR study were used. RADAR has been financially supported by main grants from the Netherlands Organisation for Scientific Research (GB-MAGW 480-03-005, GB-MAGW 480-08-006), and Stichting Achmea Slachtoffer en Samenleving (SASS), a grant from the Netherlands Organisation for Scientific Research to the Consortium Individual Development (CID; 024.001.003), and various other grants from the Netherlands Organisation for Scientific Research, the VU University Amsterdam, and Utrecht University.

Conflict of interest

The authors report no conflict of interest.

Acknowledgements

We would like to thank Olga Chelnokova for kindly providing us with the Oslo Faces database.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.euroneuro.2020.08.008.

References

Al-Ayadhi, L.Y., 2005. Altered oxytocin and vasopressin levels in autistic children in Central Saudi Arabia. Neurosciences.

Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., Riem, M.M.E., Tops, M., Alink, L.R.A., 2012. Oxytocin decreases handgrip force in reaction to infant crying in females without harsh parenting experiences. Soc. Cogn. Affect. Neurosci. 7 (8), 951–957. doi:10.1093/scan/nsr067.

Barr, D.J., Levy, R., Scheepers, C., Tily, H.J., 2013. Random effects structure for confirmatory hypothesis testing: keep it maximal. J. Mem. Lang. 68 (3), 255–278. doi:10.1016/j.jml.2012.11.001.

Bartz, J.A., Zaki, J., Ochsner, K.N., Bolger, N., Kolevzon, A., Ludwig, N., Lydon, J.E., 2010. Effects of oxytocin on recollections of maternal care and closeness. Proc. Natl. Acad. Sci. 107, 21371–21375. doi:10.1073/pnas.1012669107.

Beery, A.K., 2015. Antisocial oxytocin: complex effects on social behavior. Curr. Opin. Behav. Sci. 6 (Table 1), 174–182. doi:10.1016/j.cobeha.2015.11.006.

Beery, A.K., McEwen, L.M., MacIsaac, J.L., Francis, D.D., Kobor, M.S., 2016. Natural variation in maternal care and cross-tissue patterns of oxytocin receptor gene methylation in rats. Horm. Behav. 77, 42–52. doi:10.1016/j.yhbeh.2015.05.022.

Bernstein, D., Fink, L., 1998. Manual for the childhood trauma questionnaire. Psychol. Corp.

Bernstein, D.P., Stein, J.A., Newcomb, M.D., Walker, E., Pogge, D., Ahluvalia, T., Zule, W., 2003. Development and validation of a brief screening version of the childhood trauma questionnaire. Child Abuse Negl. doi:10.1016/S0145-2134(02)00541-0.

Bird, A., 2007. Perceptions of epigenetics. Nature 447, 396–398.

Bos, P.A., Hermans, E.J., Ramsey, N.F., Van Honk, J., 2012. The neural mechanisms by which testosterone acts on interpersonal trust. Neuroimage 61 (3), 730–737. doi:10.1016/j.neuroimage.2012.04.002.

Bos, P.A., Panksepp, J., Bluthé, R.-M., Van Honk, J., 2012. Acute effects of steroid hormones and neuropeptides on human social-emotional behavior: a review of single administration studies. Front. Neuroendocrinol. 33 (1), 17–35. doi:10.1016/j.yfrne.2011.01.002.

Brietzke, E1, Kauer Sant'anna, M, Jackowski, A, Grassi-Oliveira, R, Bucker, J, Zugman, A, Mansur, RB, B., R., 2012. Impact of childhood stress on psychopathology. Rev. Bras. Psiquiatr. 34 (4), 480-488. doi:10.1016/j.rbp.2012.04.009.

Cao, J., Liu, Q., Li, Y., Yang, J., Gu, R., Liang, J., Liu, X., 2017. Cognitive behavioural therapy attenuates the enhanced early facial stimuli processing in social anxiety disorders: an ERP investigation. Behav. Brain Funct. 1, 12. doi:10.1186/s12993-017-0130-7.

Carr, C.P., Martins, C.M.S., Stingel, A.M., Lemgruber, V.B., Juruena, M.F., 2013. The role of early life stress in adult psychiatric disorders: A systematic review according to childhood trauma subtypes. J. Nerv. Ment. Dis. 12, 1007–1020. doi:10.1097/NMD.0000000000000049.

Champagne, F.A., 2011. Maternal imprints and the origins of variation. Horm. Behav. 60, 4–11. doi:10.1016/j.yhbeh.2011.02.016.

Chatzittofis, A., Nordström, P., Uvnäs-Moberg, K., Åsberg, M., Jokinen, J., 2014. CSF and plasma oxytocin levels in suicide attempters, the role of childhood trauma and revictimization. Neuroendocrinol. Lett 35, 213–217.

Chen, X., Nishitani, S., Haroon, E., Smith, A.K., Rilling, J.K., 2019.

OXTR methylation modulates exogenous oxytocin effects on human brain activity during social interaction. Genes Brain Behav. 1, e12555. doi:10.1111/gbb.12555.

Churches, O., Wheelwright, S., Baron-Cohen, S., Ring, H., 2010. The N170 is not modulated by attention in autism spectrum conditions. Neuroreport 6, 399–403. doi:10.1097/WNR.0b013e328334311b.

Costa, B., Pini, S., Gabelloni, P., Abelli, M., Lari, L., Cardini, A., Martini, C., 2009. Oxytocin receptor polymorphisms and adult attachment style in patients with depression. Psychoneuroendocrinology 34 (10), 1506–1514. doi:10.1016/j.psyneuen.2009.05.006.

De Bellis, M. D., Hooper, S. R., Sapia, J. L., Vasterling, J. J., & Brewin, C. R. (2005). Early trauma exposure and the brain. In Neuropsychology of PTSD: Biological, *Cognitive*, and *Clinical Perspectives*. The Guilford Press. (pp. 153–177). Retrieved from http://search.ebscohost.com/login.aspx?direct=true&db=psyh&AN=2005-08636-007&site=ehost-live

Dupont, C., Armant, D., Brenner, C., 2009. Epigenetics: definition, mechanisms and clinical perspective. Semin. Reprod. Med. 27 (5), 351–357.

Ebert, A., Kolb, M., Heller, J., Edel, M.-A., Roser, P., Brüne, M., 2013. Modulation of interpersonal trust in borderline personality disorder by intranasal oxytocin and childhood trauma. Soc. Neurosci. 8 (4), 305–313. doi:10.1080/17470919.2013.807301.

Ebner, N., Lin, T., Muradoglu, M., Weir, D., Plasencia, G., Lillard, T., Connelly, J., 2018. Associations between oxytocin receptor gene (OXTR) methylation, plasma oxytocin, and attachment across adulthood. Int. J. Psychophysiol. doi:10.1016/j.ijpsycho.2018.01.008.

Ein-Dor, T., Verbeke, W.J.M.I., Mokry, M., Vrticka, P., 2018. Epigenetic modification of the oxytocin and glucocorticoid receptor genes is linked to attachment avoidance in young adults. Attach. Hum. Dev. 20 (4), 439–454. doi:10.1080/14616734.2018.1446451.

Endendijk, J.J., Spencer, H., van Baar, A.L., Bos, P.A., 2018. Mothers' neural responses to infant faces are associated with activation of the maternal care system and observed intrusiveness with their own child. Cogn. Affect. Behav. Neurosci. 18 (4), 609–621. doi:10.3758/s13415-018-0592-6.

Fani, N., Bradley-Davino, B., Ressler, K.J., McClure-Tone, E.B., 2011. Attention bias in adult survivors of childhood maltreatment with and without posttraumatic stress disorder. Cogn. Ther. Res. 35 (1), 57–67. doi:10.1007/s10608-010-9294-2.

Feuerriegel, D., Churches, O., Hofmann, J., Keage, H.A.D., 2015. The N170 and face perception in psychiatric and neurological disorders: a systematic review. Clin. Neurophysiol. 6, 1141–1158. doi:10.1016/j.clinph.2014.09.015.

Fraedrich, E.M., Lakatos, K., Spangler, G., 2010. Brain activity during emotion perception: the role of attachment representation. Attach. Hum. Dev. doi:10.1080/14616731003759724.

Glaesmer, H., Schulz, A., Häuser, W., Freyberger, H., Brähler, E., Grabe, H.J., 2013. The childhood trauma screener (CTS) - development and validation of cut-off-scores for classificatory diagnostics. Psychiatr. Prax. doi:10.1055/s-0033-1343116.

Gouin, J., Zhou, Q., Booij, L., Boivin, M., Côté, S., Hébert, M., Vitaro, F., 2017. Associations among oxytocin receptor gene (OXTR) DNA methylation in adultood, exposure to early life adversity, and childhood trajectories of anxiousness. Sci. Rep. 7.

Gratton, G., Coles, M.G.H., Donchin, E., 1983. A new method for off-line removal of ocular artifact. Electroencephalogr. Clin. Neurophysiol. doi:10.1016/0013-4694(83)90135-9.

Haas, B., Filkowski, M., Cochran, R., Denison, L., Ishak, A., Nishitani, S., Smith, A., 2016. Epigenetic modification of OXT and human sociability. Proc. Natl. Acad. Sci. U. St. A. 113 (27), E3816–E3823. doi:10.1073/pnas.1602809113.

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. U. St. A. 113 (27), E3816–E3823. doi:10.1073/pnas.1602809113.

Heim, C., Young, L.J., Newport, D.J., Mletzko, T., Miller, A.H., Nemeroff, C.B., 2009. Lower CSF oxytocin concentrations in women with a history of childhood abuse. Mol. Psychiatry 14 (10), 954–958.

Heinrichs, M., Baumgartner, T., Kirschbaum, C., Ehlert, U., 2003. Social support and oxytocin interact to suppress cortisol and subjective responses to psychosocial stress. Biol. Psychiatry 54 (12), 1389–1398.

Herrington, J.D., Riley, M.E., Grupe, D.W., Schultz, R.T., 2015. Successful face recognition is associated with increased prefrontal cortex activation in autism spectrum disorder. J. Autism Dev. Disord. doi:10.1007/s10803-014-2233-4.

Hinojosa, J.A., Mercado, F., Carretié, L., 2015. N170 sensitivity to facial expression: a meta-analysis. Neurosci. Biobehav. Rev. doi:10.1016/j.neubiorev.2015.06.002.

Hoge, E., Pollack, M., Kaufman, R., Zak, P., Simon, N., 2008. Oxytocin levels in social anxiety disorder. CNS Neurosci. Ther. 14, 165–170.

Huffmeijer, R., Alink, L.R.A., Tops, M., Grewen, K.M., Light, K.C., Bakermans-Kranenburg, M.J., van IJzendoorn, M.H., 2013. The impact of oxytocin administration and maternal love withdrawal on event-related potential (ERP) responses to emotional faces with performance feedback. Horm. Behav. doi:10.1016/j.yhbeh.2012.11.008.

Kader, F., Ghai, M., 2017. DNA methylation-based variation between human populations. Mol. Genet. Genomics doi:10.1007/s00438-016-1264-2.

Kolassa, I.T., Miltner, W.H.R., 2006. Psychophysiological correlates of face processing in social phobia. Brain Res. doi:10.1016/j.brainres.2006.08.019.

Kraaijenvanger, E.J., He, Y., Spencer, H., Smith, A.K., Bos, P.A., Boks, M.P., 2019. Epigenetic variability in the human oxytocin receptor (OXTR) gene: a possible pathway from early life experiences to psychopathologies. Neurosci. Biobehav. Rev. 96, 127–142. doi:10.1016/j.neubiorev.2018.11.016.

Kusui, C., Kimura, T., Ogita, K., Nakamura, H., Matsumura, Y., Koyama, M., Murata, Y., 2001. DNA methylation of the human oxytocin receptor gene promoter regulates tissue-specific gene suppression. Biochem. Biophys. Res. Commun. 289 (3), 681–686.

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2014). lmerTest: Tests for random and fixed effects for linear mixed effect models (lmer objects of lme4 package). R Package Version, p. R package version 2.0-6. http://CRAN.R-project.org/package=lmerTest

Laeger, I., Dobel, C., Dannlowski, U., Kugel, H., Groteged, D., Kissler, J., Zwanzger, P., 2012. Amygdala responsiveness to emotional words is modulated by subclinical anxiety and depression. Behav. Brain Res. 233 (2), 508–516.

Lancaster, K., Goldbeck, L., Puglia, M.H., Morris, J.P., Connelly, J.J., 2018. DNA methylation of OXTR is associated with parasympathetic nervous system activity and amygdala morphology. Soc. Cogn. Affect. Neurosci. 11, 1155–1162.

Lenth, R., 2016. Least-squares means: the R package lsmeans. J. Stat. Softw 1, 1–33.

Maccari, S., Krugers, H.J., Morley-Fletcher, S., Szyf, M., Brunton, P.J., 2014. The consequences of early-life adversity: neurobiological, behavioural and epigenetic adaptations. J. Neuroendocrinol. doi:10.1111/jne.12175.

Martins, C.M.S., Tofoli, S.M., de, C., Baes, C., von, W., Juruena, M., 2011. Analysis of the occurrence of early life stress in adult psychiatric patients: a systematic review. Psychol. Neurosci. doi:10.3922/j.psns.2011.2.007.

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 doi:10.1186/s12888-018-1740-9.

McGowan, P.O., Sasaki, A., D'Alessio, A.C., Dymov, S., Labonté, B., Szyf, M., Meaney, M.J., 2009. Epigenetic regulation of the glucocorticoid receptor in human brain associates with childhood abuse. Nat. Neurosci. 12 (3), 342–348. doi:10.1038/nn.2270.

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

Milaniak, I., Cecil, C.A.M., Barker, E.D., Relton, C.L., Gaunt, T.R., McArdle, W., Jaffee, S.R., 2017. Variation in DNA methylation of the oxytocin receptor gene predicts children's resilience to prenatal stress. Dev. Psychopathol. doi:10.1017/S0954579417001316.

Montalan, B., Caharel, S., Personnaz, B., Le Dantec, C., Germain, R., Bernard, C., Rebaï, M., 2008. Sensitivity of N170 and late positive components to social categorization and emotional valence. Brain Res. doi:10.1016/j.brainres.2008.07.055.

Nawijn, L., Krzyzewska, I.M., van Zuiden, M., Henneman, P., Koch, S., Mul, A.N., Olff, M., 2018. Oxytocin receptor gene methylation in male and female PTSD patients and trauma-exposed controls. Eur. Neuropsychopharmacol. doi:10.1016/j.euroneuro.2018.10.006.

Nishina, K., Takagishi, H., Fermin, A.S.R., Inoue-Murayama, M., Takahashi, H., Sakagami, M., Yamagishi, T., 2018. Association of the oxytocin receptor gene with attitudinal trust: role of amygdala volume. Soc. Cogn. Affect. Neurosci. doi:10.1093/scan/nsy075.

Noll, L.K., Mayes, L.C., Rutherford, H.J.V., 2012. Investigating the impact of parental status and depression symptoms on the early perceptual coding of infant faces: an event-related potential study. Soc. Neurosci. doi:10.1080/17470919.2012.672457.

Oitzl, M.S., Champagne, D.L., van der Veen, R., de Kloet, E.R., 2010. Brain development under stress: hypotheses of glucocorticoid actions revisited. Neurosci. Biobehav. Rev. 34, 853–866. doi:10.1016/j.neubiorev.2009.07.006.

Peltola, M.J., Strathearn, L., Puura, K., 2018. Oxytocin promotes face-sensitive neural responses to infant and adult faces in mothers. Psychoneuroendocrinology doi:10.1016/j.psyneuen.2018.02.012.

Perkeybile, A.M., Carter, C.S., Wroblewski, K.L., Puglia, M.H., Kenkel, W.M., Lillard, T.S., Connelly, J.J., 2018. Early nurture epigenetically tunes the oxytocin receptor. Psychoneuroendocrinology doi:10.1016/j.psyneuen.2018.08.037.

Proverbio, A.M., Brignone, V., Matarazzo, S., Del Zotto, M., Zani, A., 2006. Gender and parental status affect the visual cortical response to infant facial expression. Neuropsychologia doi:10.1016/j.neuropsychologia.2006.06.015.

Proverbio, A.M., Riva, F., Martin, E., Zani, A., 2010. Face coding is bilateral in the female brain. PLoS One doi:10.1371/journal.pone.0011242.

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 doi:10.1038/s41398-018-0159-x.

Puglia, M., Lillard, T., Morris, J., Connelly, J., 2015. Epigenetic modification of the oxytocin receptor gene influences the perception of anger and fear in the human brain. Proc. Natl. Acad. Sci. U. S. A. 112 (11), 3308–3313. doi:10.1073/pnas.1422096112.

Ratner, K.G., Amodio, D.M., 2013. Seeing "us vs. them": minimal group effects on the neural encoding of faces. J. Exp. Soc. Psychol. doi:10.1016/j.jesp.2012.10.017.

Riem, M.M.E., De Carli, P., van IJzendoorn, M.H., Linting, M., Grewen, K.M., Bakermans-Kranenburg, M.J., 2017. Emotional maltreatment is associated with atypical responding to stimulation of endogenous oxytocin release through mechanically-delivered massage in males. Psychoneuroendocrinology doi:10.1016/j.psyneuen.2017.08.017.

Rockliff, H., Karl, A., McEwan, K., Gilbert, J., Matos, M., Gilbert, P., 2011. Effects of intranasal oxytocin on "compassion focused imagery. Emotion 11 (6), 1388–1396. doi:10.1037/a0023861.

Rokicki, J., Kaufmann, T., van der Meer, D., Alnæs, D., Westlye, L.T., Quintana, D.S., Dieset, I., 2019. Oxytocin pathway gene networks in the human brain. Nat. Commun. doi:10.1038/s41467-019-08503-8.

Rubin, L., Connelly, J., Reilly, J., Carter, C., Drogos, L., Pournajafi-Nazarloo, H., Sweeney, J., 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 1 (2), 141–151. doi:10.1016/j.bpsc.2015.10.003.

Schmidt, S., Mohr, A., Miltner, W., Straube, T., 2010. Task-dependent neural correlates of the processing of verbal threat-related stimuli in social phobia. Biol. Psychol. 84, 304–312.

Schneider-Hassloff, H., Straube, B., Jansen, A., Nuscheler, B., Wemken, G., Witt, S.H., Kircher, T., 2016. Oxytocin receptor polymorphism and childhood social experiences shape adult personality, brain structure and neural correlates of mentalizing. Neuroimage doi:10.1016/j.neuroimage.2016.04.009.

Shackman, J.E., Shackman, A.J., Pollak, S.D., 2007. Physical abuse amplifies attention to threat and increases anxiety in children. Emotion 7 (4), 838–852. doi:10.1037/1528-3542.7.4.838.

Simons, R., Lei, M., Beach, S., Cutrona, C., Philiber, R., 2017. Methylation of the oxytocin receptor gene mediates the effect of adversity on negative schemas and depression. Dev. Psychopathol. 29 (3), 725–736.

Smearman, E., Almli, L., Conneely, K., Brody, G., Sales, J., Bradley, B., Smith, A., 2016. Oxytocin receptor genetic and epigenetic variations: association with child abuse and adult psychiatric symptoms. Child Dev. 87 (1), 122–134. doi:10.1111/cdev.12493.

Spencer, H., Heitland, I., Montoya, E.R., Branje, S.J.T., Bos, P.A., 2018. Ethnic effects when facing children: an ERP study. Biol. Psychol. doi:10.1016/j.biopsycho.2018.08.015.

Szyf, M., Bick, J., 2012. DNA methylation: a mechanism for embedding early life experiences in the genome. Child Dev. 84, 49–57.

Team, R. (2013). *R: a language and environment for statistical computing*.

Teicher, M.H., Samson, J.A., Anderson, C.M., Ohashi, K., 2016. The effects of childhood maltreatment on brain structure, function and connectivity. Nat. Rev. Neurosci. doi:10.1038/nrn.2016.111.

Tops, M., Van Peer, J.M., Korf, J., Wijers, A.A., Tucker, D.M., 2007. Anxiety, cortisol, and attachment predict plasma oxytocin. Psychophysiology doi:10.1111/j.1469-8986.2007.00510.x.

Tottenham, N., 2012. Human amygdala development following early-life stress. Neuropsychopharmacology 38, S5. doi:10.1038/npp.2012.218.

Wojdacz, T.K., Dobrovic, A., 2007. Methylation-sensitive high resolution melting (MS-HRM): a new approach for sensitive and high-throughput assessment of methylation. Nucleic Acids Res. doi:10.1093/nar/gkm013.

Ziegler, C., Dannlowski, U., Bräuer, D., Stevens, S., Laeger, I., Wittmann, H., Domschke, K., 2015. Oxytocin receptor gene methylation: converging multilevel evidence for a role in social anxiety. Neuropsychopharmacology 40 (6), 1528–1538. doi:10.1038/npp.2015.2.