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Salivary oxytocin mediates the association between emotional maltreatment and responses to emotional infant faces

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HIGHLIGHTS

- Emotional maltreatment is positively associated with oxytocin
- OXTR rs53576 does not moderate relation between emotional maltreatment and oxytocin
- Salivary oxytocin is associated with positive evaluation of happy expressions
- Maltreatment indirectly influences responses to happy faces by modulating oxytocin

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ABSTRACT

Childhood emotional maltreatment has been associated with a higher risk for maltreating one's own offspring. In the current study, we explored a possible role of oxytocin in mediating the association between childhood emotional maltreatment and participants' interpretation of infant facial expressions. Oxytocin levels were measured in 102 female participants using saliva samples. They rated the mood of thirteen infants with happy, sad and neutral facial expressions. Emotional maltreatment indirectly influenced responses to happy infant faces by modulating oxytocin levels: higher self-reported emotional maltreatment was related to higher levels of salivary oxytocin which were in turn related to a more positive evaluation of happy infant expressions, but not to the evaluation of sad infant expressions. Oxytocin receptor polymorphism *rs53576* did not moderate the relation between maltreatment experiences and salivary oxytocin levels. Early emotional maltreatment might indirectly affect emotional information processing by altering the oxytonergic system.

1. Introduction

Childhood emotional maltreatment has a worldwide prevalence of 27% [1], and it has enduring effects on neural, physiological and behavioral development [2–4]. Emotional maltreatment has been associated with increased vulnerability to mental health problems such as depression and anxiety, and antisocial behavior [5,6]. In parenting, it might also lead to negative appraisal of infant signals [7] and a higher risk for maltreating one's own offspring [8–11]. In the current study, we explored a possible role of oxytocin in mediating the association between childhood emotional maltreatment and participants' evaluation of infants' mood as derived from infants' facial emotional expressions.

Oxytocin is a neuro-peptide associated with trust, empathy and emotion recognition [12,13]. It is well known for its anxiolytic effects and its effects on prosocial behaviors such as in-group favoritism [14] and sensitive parenting [15–17]. Oxytocin levels can be affected by stressful early life experiences [18,19]. However, the direction of effects is unclear. One study demonstrated a negative relation between early life stress and oxytocin levels, with more stress leading to lower levels of oxytocin [18] whereas other studies showed a positive relation [19]. The diverging outcomes might be (partly) explained by technical differences between these studies, as the oxytocin levels were measured in different body fluids, e.g. in the cerebrospinal fluid in the study of Heim et al. [18] and in plasma in the study of Pierrehumbert et al. [19]. In the present study, we assessed oxytocin levels in saliva. Salivary

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oxytocin levels have been found to be correlated to plasma oxytocin levels (although modestly; for a review see [20]) and associated with parent and child's social engagement, affect synchrony and positive communication sequences [21]. We examined the relation between early life emotional maltreatment experiences and salivary oxytocin levels. Moreover, we tested a possible moderating role of oxytocin receptor polymorphism (OXTR) *rs53576* in the relation between emotional maltreatment and oxytocin levels [22]. In previous studies OXTR *rs53576* has been suggested to be a moderator of the effects of intranasal oxytocin administration on the preferences for infant faces as compared to adult faces. Intranasal oxytocin increased the preference for infant faces, but this effect was present only for individuals with GG genotype [22].

Using intranasal administration, oxytocin has been mostly investigated for its role in facial emotion recognition (for meta analysis see [12]). Although most of these studies show increased recognition of happy faces [23], some also report increased recognition of other emotions such as anger and fear [24]. To our knowledge, no studies have examined whether individual differences in endogenous levels of oxytocin are associated with differences in emotion recognition. Moreover, none of the previous studies assessed the association of oxytocin with recognition of emotional *infant* faces as opposed to adult faces. From an evolutionary perspective, oxytonergic functioning is strongly implicated in parent–infant bonding. For example, oxytocin selectively increases the preference for infant faces as compared to adult faces [16,22]. In the present study we used images of sad and happy infants to test whether salivary oxytocin levels were related to the evaluation of infant mood as derived from infants' facial emotional expressions.

Emotional maltreatment has been associated with processing biases for some negative emotional stimuli such as anger and sadness, yielding heightened responsivity and reactivity [25–28]. Impaired emotion recognition might result in more problematic interpersonal relationships [29] and more insensitive behavior towards offspring. We therefore examined the involvement of the oxytonergic system in the underlying mechanism responsible for the processing bias of emotional information in individuals with emotional maltreatment experiences.

To summarize, we hypothesized that childhood experiences of emotional maltreatment would be associated with oxytocin levels. However, given the contradictory findings from previous studies, we were not able to predict whether more maltreatment experiences would lead to higher or to lower oxytocin levels. Furthermore, we explored the potential role of OXTR *rs53576* in moderating the relation between emotional maltreatment and oxytocin levels. Moreover, in line with previous studies with intranasal administration, we hypothesized that for women with higher levels of oxytocin, infant emotional expressions would be more salient than for women with lower levels of oxytocin. Furthermore, we tested whether oxytocin mediated any association between childhood maltreatment experiences and mood ratings as derived from infants' facial expressions.

2. Methods

2.1. Participants and procedure

Three hundred and fifty three undergraduate students from the Leiden University departments of Education and Child studies and Psychology completed online questionnaires about their childhood experiences of abuse and neglect. Of these, 102 healthy female students (age $M = 19.86$ years, $SD = 1.42$) were randomly invited to participate in the present study. All were nulliparous, a majority (85%) of the participants reported being in the luteal phase of their menstrual cycle (third or fourth week following menstruation) and 74% of the participants reported using oral contraceptives. Exclusion criteria were use of steroidal or any other interfering medications such as analgesics and anti-inflammatory drugs. All participants were non-smokers and reported not having used any recreational drugs in the six months before the experiment. Participants reported not having any current or past neurological or psychological disorder(s). Two participants were excluded from the analysis due to technical errors in the computer session. Seven participants were not included in the final analysis because of missing values of salivary oxytocin levels. The study was approved by the Leiden University Medical Center ethics committee.

Participants were invited to the laboratory for a computer session. The sessions started at 0900, 1200 or 1500 h. Participants provided saliva samples that were used to determine oxytocin levels. Next, they rated the emotion of images of faces of thirteen infants with happy, sad and neutral facial expressions. Participants also rated several infant faces following this task, which is described elsewhere [30].

2.2. Task and measures

2.2.1. Stimuli

The stimuli consisted of grayscale pictures of thirteen infants, each of which was shown with neutral, happy and sad expressions. Infant images were obtained from a standardized database (for detailed description of the stimuli see [31]). Faces were all forward facing, with direct eye gaze, matched for size (300 × 300 pixels) and luminosity. Participants saw a facial image at the center of a 15.3 inch monitor with a visual analog scale immediately to the right. The participants were instructed to rate the mood of the presented picture from *very positive* to *very negative*. The scale ranged from 4 to −4.

Reliability analyses of the mood ratings of the neutral, happy and sad infant faces showed that the internal consistency for the happy and sad faces was high, Cronbach's α (happy) = .81; Cronbach's α (sad) = .84. However, the internal consistency for neutral faces' mood rating was low (Cronbach's $\alpha = .49$); responses to neutral facial expressions were therefore not analyzed. Two scales, one for the mood rating of the happy facial expression and one for the mood rating of the sad facial expression, were created by averaging the ratings for the thirteen infants. The scale for sad facial expression was reversed so that a higher value would represent a more negative rating.

2.2.2. Salivary oxytocin

The saliva samples were immediately stored at −20 °C until batch assay. The samples were assayed using the standard procedures described in detail elsewhere, using the commercially available Enzyme Immuno Assay (EIA) kit (ADI-900-153, Enzo Life Science, Plymouth Meeting, PA) [32–34]. An extraction step was performed to concentrate the sample, increase precision and reduce matrix interference. A strata-X 33 μm polymeric reversed phase SPE sorbent was equilibrated in a 96-well plate containing 60 mg sorbent per well, Phenomenex, Torrance CA, by adding 1 ml MeOH followed by 1 ml of water. Next, 0.8 ml of saliva was acidified with 0.4 ml of 1.5% trifluoroacetic acid (TFA) and centrifuged at 6000 ×*g* for 20 min at 4 °C. The supernatant was loaded onto the pre-treated strata-X plate. The wells were slowly washed with 1.5 ml of 0.1% TFA, and then the peptide was eluted with 1 ml of 80% acetonitrile. The eluant was collected in a polystyrene tube and evaporated to dryness under a N_2 stream. The residue was reconstituted in 250 μl of assay buffer. Oxytocin extraction efficiency was 94%, as determined by spiking with a known amount of hormone and extracting this known amount along with the samples. Oxytocin levels in extracted saliva were then quantified using the oxytocin EIA, in which the endogenous oxytocin hormone competes with exogenously added alkaline phosphatase linked oxytocin, for binding sites on oxytocin antibody. After overnight incubation at 4 °C, excess reagents were washed away and the bound oxytocin phosphatase was incubated with a substrate. After 1 h this enzyme reaction, which generates a yellow color, was stopped and the optical density (OD) was measured on a Sunrise plate reader (Tecan, Research Triangle Park, NC) at 405 nm. The intensity of the color is inversely proportional to the concentration of endogenous oxytocin. The hormone content (in pg/ml) was determined by plotting the OD of each sample