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Effects of intranasal oxytocin administration on memory for infant cues: Moderation by childhood emotional maltreatment

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Abstract

Oxytocin has been implicated in parent-infant attachment and social recognition. With respect to emotion recognition memory, both memory enhancing and memory impairing effects have been observed, suggesting an influence of individual factors on oxytocin effects. Despite its crucial role in parent-infant attachment, the effects of oxytocin on memory for infant cues have not been studied so far. In the present study, we assessed the effects of oxytocin on memory for infant temperamental cues, and we assessed whether these effects are moderated by childhood emotional maltreatment. Hundred and two healthy nulliparous females participated in a randomized, double-blind, between subjects experimental study with intranasal oxytocin or placebo administration. Experiences with emotional maltreatment before age 16 were self-reported using the Childhood Trauma Questionnaire. Participants’ memory for positive and negative infant cues was tested using the Baby Social Reward Task, a probabilistic learning task with experimentally manipulated temperament of infant faces. Participants who reported more childhood emotional maltreatment made more recognition mistakes in distinguishing happy infants from sad ones after intranasal oxytocin administration. They were less accurate for infant pairs that were derived from the pairs in the learning task, but not for the pairs they were trained on. More specifically, they made more mistakes on the ‘high conflict’ pairs, with pairings that were more similar and thus more difficult to distinguish. We found evidence for amnesic effects of oxytocin in a decision making task, for infant stimuli in individuals with more experiences of emotional maltreatment. Our findings also suggest that the effects of oxytocin may depend on the uncertainty of the information being processed.

Keywords

Oxytocin, Intranasal administration, Emotional maltreatment, Memory, Decision-making, Baby Social Reward Task
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Introduction

Oxytocin is considered a sociability hormone; it has been shown to elevate trust in and empathy for conspecifics (De Dreu et al., 2010; Kosfeld et al., 2005; For a meta-analysis see Van IJzendoorn and Bakermans-Kranenburg, 2012a). Oxytocin has also been widely implicated in parent-infant attachment. High postpartum levels of oxytocin have been associated with parents’ synchronized and sensitive behavior towards their infants (Feldman et al., 2011; Feldman et al., 2007). Intranasal administration of oxytocin has been shown to increase sensitive caretaking behavior in parents (Naber et al., 2013) and to reduce aversion in response to infant crying as indicated by decreased amygdala activity (Riem et al., 2011).

Next to these prosocial and parent-infant bonding effects, oxytocin has also been implicated in social memory processes (for reviews see Guastella and MacLeod, 2012; Heinrichs et al., 2009; Striepens et al., 2011). The reported effects of oxytocin in memory are however not unequivocal. Whereas some studies have shown enhanced memory after intranasal oxytocin administration (Guastella et al., 2008; Rimmele et al., 2009; Savaskan et al., 2008), other studies have shown impaired memory (Heinrichs et al., 2004; Herzmann et al., 2012). The evidence for effects of intranasal oxytocin on emotion recognition has also been mixed. Oxytocin administration either increased recall of happy but not angry or neutral faces (Guastella et al., 2008), or improved recognition for angry and neutral, but not for happy faces (Savaskan et al., 2008). One study showed valence independent effects of oxytocin on facial memory, but no effect for non-social stimuli (Rimmele et al., 2009). More recently, amnesic effects of oxytocin for both social and non-social visual objects have been reported (Herzmann et al., 2012). Amnesic effects of oxytocin have also been shown for reproduction related words as compared to neutral words (Heinrichs et al., 2004).

Most of the research on oxytocin effects on memory processes has been conducted using adult facial stimuli. Infant cues may be processed differently compared to adults’ (Kringelbach et al., 2008). Infant faces are preferred over adult faces and oxytocin has been shown to further increase this preference for infant faces (Marsh et al., 2012). Moreover, infant cues are related to higher activation in reward pathways that interact with the oxytonergic system (Glocker et al., 2009; Riem et al., 2011; Strathearn et al., 2009). Altogether these findings indicate that oxytocin administration may have different effects on memory processes when infant stimuli are used. In the present study we used intranasal oxytocin administration to examine effects on memory for infant temperamental
cues. In order to increase the ecological validity of the paradigm, we used a combination of facial and auditory cues, with a laughing sound accompanying smiling facial expressions and a crying sound accompanying sad facial expressions.

Recent research suggests an important role for individual factors moderating the effects of oxytocin on behavioral and neurobiological responses (for a review see MacDonald, 2013). Apart from gender differences (Fischer-Shofty et al., 2012) and genetic factors (e.g. oxytocin receptor gene; Marsh et al., 2012), attachment style has been suggested as a potential moderator of oxytocin effects. After intranasal administration of oxytocin less anxiously attached individuals remembered their mother as more caring and more close whereas more anxiously attached individuals remembered their mother as less caring and less close (Bartz et al., 2010). Moreover, childhood experiences with harsh discipline or emotional maltreatment may modulate the influence of oxytocin on behavior. Parental use of love withdrawal has been shown to moderate the effect of oxytocin on generosity, pro-social behaviors and behavioral responses to infant crying (Bakermans-Kranenburg et al., 2012; Riem et al., 2013; Van IJzendoorn et al., 2011). In the present study we assessed whether experiences of emotional maltreatment during childhood moderated the effects of oxytocin on memory for infant cues.

Emotional maltreatment is increasingly identified as negatively impacting on long-term psychological functioning, with effects comparable to or even stronger than other forms of maltreatment. The prevalence of emotional maltreatment is high. In the Netherlands, 26 per 1000 individuals were reported being emotionally abused or neglected (Euser et al., internal report). Emotional maltreatment has been associated with behavior problems such as internalizing and externalizing problems, social impairment, low self-esteem, and psychiatric illnesses (Stange et al., 2013; Valentino et al., 2009; Van Harmelen et al., 2010a). With respect to cognitive development, children with experiences of emotional maltreatment showed heightened false recognition memory (Cicchetti et al., 2010). Childhood emotional abuse has also been associated with lower oxytocin levels measured in cerebrospinal fluid (Heim et al., 2008). Moreover, childhood emotional maltreatment is associated with differential neural processing of emotions, for example, greater amygdala activity in response to emotional faces (Van Harmelen et al., 2012). Childhood emotional maltreatment may thus interact with the effects of oxytocin, as oxytocin is also associated with altered amygdala activity in response to emotional infant stimuli (Riem et al., 2011).

In the current study we assessed the effects of intranasal oxytocin administration
on memory for infant temperamental cues, and we examined potential moderation of these effects by experiences of emotional maltreatment during childhood. After oxytocin or placebo administration, participants performed a probabilistic social learning task, referred to as the “Baby Social Reward Task” (BSRT; Parsons et al, in press), in which participants learned about ‘sad’ and ‘happy’ infants, and were later tested to see if they could correctly differentiate happy infants from sad ones. We hypothesized that intranasal oxytocin would affect the performance of the participants and that this effect would be moderated by experiences of emotional maltreatment. Because earlier reports are equivocal in terms of the direction of the oxytocin effects, (memory enhancing versus amnesic) and about the role of valence (happy versus angry and neutral facial expressions) we did not further specify the direction of our hypotheses.

Methods

Participants

Three hundred and fifty three undergraduate students from the Institute of Education and Child Studies, Leiden University completed questionnaires about their early life traumatic experiences. Of these, 102 females (age $M = 19.86$ years, $SD = 1.42$) were randomly selected to participate in the present study. All were nulliparous, 85% reported being in the luteal phase of their menstrual cycle and 74% of the participants used oral contraceptives. Exclusion criteria included pregnancy, breastfeeding and 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). Three participants were excluded from the analysis due to technical errors in the computer session. Fifty-three participants (age $M = 19.75$, $SD = 1.08$) received oxytocin and 46 participants (age $M = 19.94$, $SD = 1.74$) received placebo, intranasally. In the oxytocin group, 43 participants received 16 IU dose and ten received a higher volume dose of 24 IU. These different doses of oxytocin have been shown to make no difference in salivary oxytocin levels (Van IJzendoorn et al, 2012b). Seven participants ($n = 4$ in the oxytocin condition; $n = 3$ in placebo condition) were not included in the final analysis because of missing values of basal salivary oxytocin levels. The study was approved by the Leiden University Medical Center ethics committee.
**Procedure**

Participants were invited to the laboratory for an experimental session lasting for about 2 hours. Sessions started at 0900h, 1200h or 1500h. Participants first rated six neutral infant faces on physiognomic (e.g. cuteness) and temperamental characteristics (e.g. irritable, responsive) (Parsons et al, in press). They provided saliva samples for the assessment of basal oxytocin levels. Participants rated their mood using the Positive Affect Negative Affect Scale (PANAS; Crawford and Henry, 2004). Next, they received either oxytocin or placebo (saline) nasal spray in a randomized double blind design. Forty-five minutes after nasal spray administration participants again rated the infant faces and PANAS. Supplementary information (S1 and S2) provides further details on infant characteristics and participants’ mood assessments. Approximately 55 - 60 minutes after the nasal spray they performed the BSRT (Parsons et al, in press) with experimentally manipulated temperament (happy or sad) of six different infants. A detailed description of the BSRT is provided below. All ratings and tasks were programmed and presented using Presentation software (Version 14.4 Neurobehavioral Systems, Inc., www.neurobs.com).

**Infant stimuli**

The visual stimuli consisted of the faces of six infants, each of which was shown with neutral, smiling and crying expressions. The infant images were obtained from a standardized database described in detail elsewhere (Kringelbach et al, 2008). Faces were all forward facing, with direct eye gaze, matched for size (300x300 pixels) and luminosity. Images were presented in grayscale on a 15.3 inch computer monitor. Auditory stimuli comprised twelve infant vocalizations of 1500 ms duration; six of infant crying and six of infant laughing (for a detailed description see Young et al, 2012). Vocalizations were presented at 50 dB SPL above each participant’s measured threshold, using in-ear earphones (Sennheiser CX300II).

**Childhood emotional maltreatment**

Participants indicated their experiences of abuse and neglect until the age of 16 by rating the Childhood Trauma Questionnaire Short Form (CTQ-SF; Bernstein et al, 2003). Twenty-eight items were presented to assess experiences of physical abuse, emotional abuse, sexual abuse, physical neglect, and emotional neglect. Each item (e.g. “During my childhood I felt hated by family”) was rated on a 5-point rating scale ranging from never true to very often true. In our sample of college students, the prevalence of physical and sexual abuse and neglect was rather low. Emotional abuse and neglect had a wider
range and a better distribution for this sample. An ‘Emotional Maltreatment’ scale was created by averaging the items tapping into the ‘emotional abuse’ \((M = 1.36, SD = 0.52)\) and ‘emotional neglect’ \((M = 1.61, SD = 0.56)\) dimensions. Similar scales have been used in previous studies to assess the developmental and behavioural outcomes of emotional maltreatment (Van Harmelen et al., 2012; Van Harmelen et al., 2010b). Emotional maltreatment has been defined by American Professional Society on the Abuse of Children (APSAC; Binggeli et al., 2001) as acts of commission (emotional abuse, such as degrading, terrorizing, belittling, blaming, exploiting) and/or omission (emotional neglect such as isolation, rejection, denying emotional responsiveness), which conveys to the child that he/she is worthless, unloved, and unwanted, and are harmful to the child’s emotional developmental needs. The scores on this scale ranged from 1 to 3.60. The internal consistency of this scale was high (Cronbach’s \(\alpha = .91\)). Scale scores were dichotomized into lower and higher levels of emotional maltreatment experiences using a median split (Bakermans-Kranenburg et al., 2012; Riem et al., 2013).

**Baby Social Reward Task**

Approximately 55 - 60 minutes after the nasal spray the participants performed the Baby Social Reward Task. The baby social reward task aims to manipulate the perceived temperament of a set of infant faces. The task is comprised of two parts, a training phase where the participants learn about the temperament of a series of infants by selecting an infant and receiving feedback and a testing phase measuring what they have learnt (Figure 1).

**Training.** The training phase is a probabilistic learning paradigm where three fixed pairs of infant faces are presented to the participant in a random order. At the start of each trial, a pair of neutral faces are presented, one on the top half of the computer screen and the other on the bottom half. Participants then select one of the faces and receive feedback providing information about that infants’ temperament. Feedback is in the form of a change in facial expression accompanied by an appropriate vocalization. Each pair of infants consists of a relatively 'happy' and a relatively 'sad' infant and participants come to learn which infant is which, by trial and error. The three pairs of infants vary in the probability of each infant being happy or sad. In the easiest pair, the happy infant smiles and laughs in 80% of the trials and frowns and cries in the remaining 20% of the trials. The sad infant in this pair laughs in only 20% of the trials and cries in the other 80% of trials. In the second pair, the happy infant laughs in 70% of the trials and the sad infant laughs in 30% of the trials. In the final and hardest to learn pair, the happy infant
Figure 1. The Baby Social Reward Task. (A) In the training round, participants watch a pair of neutral infant faces and are asked to select the upper or lower face by pressing the UP or DOWN keyboard key. Feedback follows their selection: The selected image is replaced with either the smiling face image of the same infant accompanied by a laughing sound, or its frowning face image accompanied by a crying sound. (B) In the testing round, participants watch a pair of neutral infant faces and are asked to select the happier infant (based on what they learnt during the training round) by pressing the UP or DOWN keyboard key. Each trial is shown for a maximum of 4000ms and is counted as ‘missed’ if the participant does not respond within this time frame. After selecting an infant participants see a fixation cross for 500 ms before the next trial begins. (Original infant faces are replaced by cartoon images, as required by the ethical committee.)
laughs in 60% of the trials while the sad infant laughs in 40% of the trials. Training was divided into 2 rounds, with 60 trials in each round (20 trials per infant pair). In one round, participants were asked to select the happy infant of each pair and in the other round they were asked to select the sad infant. This setting ensured a roughly equal exposure to the two infants of each pair. The order of training rounds (selecting the happy infant or selecting the sad infant) was counterbalanced among participants.

At the start of each trial, the participants had no idea about the happy and sad infant of each pair. The participant selected an infant by pressing the ‘up’ keyboard key for the infant presented on the top half of the computer screen and ‘down’ keyboard key for the infant presented on the bottom half of the computer screen. Visual and auditory feedback followed their selection: The selected image was replaced with either the smiling face image of the same infant accompanied by a laugh, or its frowning face image accompanied by a cry. The unselected image remained neutral during feedback. The total duration of the feedback was 1500 ms after which participants saw a fixation cross for 500 ms before the next trial started. Through repeated trials, participants could then estimate how often each infant laughed or cried and work out which infant was the happier or sadder of the pair.

The order of trials during this phase was randomized between sessions and participants. The identity of the image from each pair that appeared at the top of the screen was also randomized between trials. Further, the actual identities of which infant face was the 80% happy, 70% happy and so on were randomized between participants. This was to exclude the possibility of differences in perceived temperament or cuteness affecting the learning process. The training was self-paced and it took around 5-6 minutes for the participants to complete both training rounds.

A correct response was coded as ‘1’ and an incorrect response was coded as ‘0’. Cumulative percentage scores were created for each trial by adding the correct responses and dividing the sum by the number of trials (e.g. response: $x_1$, $(x_1 + x_2)/2$, $(x_1 + x_2 + \ldots + x_n)/n$). The participants were instructed to find the happy or sad infant by trial and error. Therefore, all the participants had some incorrect responses in the beginning. As the cumulative percentage score at a later trial takes into account all correct and incorrect responses in the previous trials, the mean average across conditions show a final learning value between 70-80% (Figure 2) even though most of the participants were performing close to 100% in the final trials.
Testing. Immediately following the training phase, in the testing phase participants were presented with random pairs (all possible combinations) of infants (e.g., 80% happy paired with 70% happy; 30% happy paired with 40% happy) and were asked to identify the happier infant of the pair. There were 15 possible pairs, and each pair was presented four times, resulting in a total of 60 trials. The pairs were presented in a random order. Similar to the training phase, the participants saw two neutral faces, one on the top and another on the bottom of the computer screen. However, unlike the training phase, participants were instructed to make the selection as quickly as possible (for assessing reaction time). Each trial was shown for a maximum of 4000ms and was counted as ‘missed’ if the participant had not responded within this time frame. In the testing phase the participants did not receive feedback following their selection (in form of change in facial expression and accompanying sound); after selecting an infant participants saw a fixation cross for 500 ms before the next trial started.

Correct responses were counted if participants chose the happier of the two infants (e.g., the 70% happy infant over the 60% happy infant). The total number of correct responses was counted for all the trials and an average overall score was computed (the scores could range from 0 to 1). To further localize the effects, trials were divided into trained or explicit pairs (the three pairs that had been presented during the training phase) and derived or implicit pairs (the other pairs).

The derived pairs were further classified based on two principles; (a) Low conflict (pairs consisting of a happy and a sad infant, e.g., 70% happy infant paired with 40% happy infant) versus High conflict (pairs consisting of two happy or two sad infants, e.g., 30% happy infant paired with 20% happy infant). (b) Based on the presence of a particular infant in the pair (e.g. all trials containing 80% happy infant) (results for this pairing are presented as supplementary information S4). The average reaction time to select an infant was also recorded for all pairings.

Statistical analyses

A series of analyses of co-variances (ANCOVAs) were performed to analyze the effects of oxytocin administration and moderation of the effect by childhood emotional maltreatment on accuracy of performance and ratings of infant characteristics. Post-hoc analyses were performed to localize significant differences between the four groups; placebo with lower levels of emotional maltreatment, placebo with higher levels of emotional maltreatment, oxytocin with lower levels of emotional maltreatment, and oxytocin
with higher levels of emotional maltreatment. For a detailed description of the analyses and covariate(s) selection see supplementary information S3.

Results

Training

Three infant pairs (80% and 20% happy, 70% and 30% happy, 60% and 40% happy) were presented in random order the training phase; each pair was presented 40 times. Figure 2 represents the cumulative percentage of correct scores for the oxytocin and placebo groups for the three infant pairs, for the higher and lower emotional maltreatment groups separately.

In order to analyze the differences in learning between the oxytocin and placebo groups, difference scores were computed by subtracting the cumulative percentage score of each trial from the cumulative percentage score of the following trial (for e.g. trial3 - trial2, trial4 – trial3). For differences occurring in the acquisition phase of the learning, the first quarter of each pair’s trials (excluding the first trial) were analyzed in a repeated measure ANOVA with trials as the within-subject factor and nasal spray and emotional maltreatment as between-subjects factors. Contraceptive use, basal salivary oxytocin level, and difference scores for negative affect and cuteness were used as covariates (see supplementary information S3). Similar analyses were performed for the last quarter of the trials in order to test for differences between the four groups in the final learning phase.

None of the main or interaction effects were significant. The four groups did not differ in the acquisition phase, or in the final phase of learning. Participants in the four groups learned to discriminate the happy and sad babies equally well.

Testing

Correct responses. Total accuracy was calculated for the 15 pairs of infants presented during the testing phase. A univariate analysis was conducted with nasal spray and emotional maltreatment conditions as fixed factors. Contraceptive use, basal salivary oxytocin levels, difference scores for negative affect and cuteness were used as covariates (see supplementary information S3). The total number of correct responses in the final stage of the training phase (final 10 trials for each infant pair) was also used as a covariate in order to control for individual differences in learning.
Figure 2. Learning curves for the training phase. Raw cumulative percentage correct scores for the lower and higher maltreated groups, with oxytocin or placebo treatment. (A) 80 - 20 Pair (B) 70 - 30 pair (C) 60 - 40 pair.
We found a significant interaction effect of nasal spray by emotional maltreatment, $F(1, 83) = 4.55; p = .036$, $\eta^2 = .052$. Post-hoc analyses showed that participants in the oxytocin group with higher emotional maltreatment scores differed significantly from participants in the placebo group with higher maltreatment scores ($p = .004$) and from participants in oxytocin group with lower maltreatment scores ($p = .024$). Participants in the oxytocin group with higher maltreatment scores had fewer correct responses ($M = 0.63, SE = 0.03$) than participants in the placebo group with higher maltreatment scores ($M = 0.74, SE = 0.03$) and participants in the oxytocin group with lower maltreatment scores ($M = 0.71, SE = 0.03$). The means of the placebo group with lower maltreatment ($M = 0.71, SE = 0.03$) did not differ significantly from any of the other three groups (Figure 3).

Next, pairs on which the participants were trained (80% – 20% happy, 70% – 30% happy, 60% – 40% happy) and pairs on which the participants had to derive associations themselves (all other pairs e.g. 70% – 40% happy pair, 30% – 20% happy pair, etc.) were examined. Mean scores on trained and derived trials were tested using a MANCOVA.
with nasal spray and emotional maltreatment as fixed factors. The set of five covariates used in the accuracy analysis were also used in this analysis. Participants in the four groups did not differ on the trained pairs. There was a significant interaction between nasal spray and emotional maltreatment for the derived pairs, $F(1, 83) = 6.23, p = .015, \eta^2 = .070$. Post-hoc analyses showed that participants in the oxytocin group with higher maltreatment scores differed significantly from participants in the placebo group with higher maltreatment scores ($p = .002$) and from participants in the oxytocin group with lower maltreatment scores ($p = .012$). Participants in the oxytocin group with higher maltreatment scores had fewer correct responses ($M = 0.60, SE = 0.03$) than the participants in the placebo group with higher maltreatment ($M = 0.72, SE = 0.03$) and from participants in the oxytocin group with lower maltreatment scores ($M = 0.69, SD = 0.03$) (Figure 4). The means of the placebo group with lower maltreatment ($M = 0.68, SE = 0.03$) did not differ significantly from any of the other three groups.

![Figure 4](image.png)

**Figure 4. Average correct responses in the testing phase on trained and derived trials.** Participants with higher emotional maltreatment scores with oxytocin administration were significantly less accurate than higher emotional maltreatment scores with placebo administration and lower emotional maltreatment scores with oxytocin administration on the derived trials. The scores could range from 0 to 1. Data are plotted as mean ± SEM. *$p < .05$*

The derived pairs were further classified into low conflict (pairs with a happy
and a sad infant, e.g. 70% – 20% happy pair) and high conflict (pairs with two happy or two sad infants, e.g. 30% – 20% happy pair, 80% – 70% happy pair). Means of low conflict and high conflict trials were tested using a MANCOVA with nasal spray and emotional maltreatment as fixed factors. The five covariates used in the previous analyses were also used in this analysis. There was a significant interaction between nasal spray and emotional maltreatment conditions for the high conflict pairs, $F(1, 83) = 4.60, p = .035, \eta^2 = .053$. Post-hoc analyses showed that participants in the oxytocin group with higher maltreatment scores differed significantly from participants in the placebo group with higher maltreatment scores ($p = .011$) and from participants in the oxytocin group with lower maltreatment scores ($p = .046$) for the high conflict trials (Figure 5). Participants in the oxytocin group with higher maltreatment scores had fewer correct responses ($M = 0.49, SD = 0.03$) than participants in the placebo group with higher maltreatment scores ($M = 0.62, SD = 0.04$) and participants in the oxytocin group with lower maltreatment scores ($M = 0.59, SD = 0.04$). The means of the placebo group with lower maltreatment ($M = 0.56, SE = 0.04$) did not differ significantly from any of the other three groups.

**Figure 5. Correct responses in the testing phase on derived trials divided on difficulty levels - low conflict and high conflict trials.** Participants with higher emotional maltreatment scores and oxytocin administration made significantly more incorrect choices as compared to either higher emotional maltreatment scores with placebo administration and lower emotional maltreatment scores with oxytocin administration on high conflict trials. The scores could range from 0 to 1. Data are plotted as mean ± SEM. * $p < .05$
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Further classification of high conflict trials into trials containing two happy infants, and trials containing two sad infants did not show any effect of the nasal spray or emotional maltreatment condition.

**Reaction times.** All analyses were repeated with reaction times as the dependent variable. There were no effects of nasal spray or emotional maltreatment on reaction times. Participants in the oxytocin and placebo groups with lower and higher maltreatment scores did not differ in the speed of selecting the happier infant.

**Discussion**

Participants who reported more childhood emotional maltreatment made more recognition mistakes in distinguishing happy infants from sad ones after intranasal oxytocin administration. This ‘amnesic’ effect was present only for the derived (or implicit) pairs and not for the trained pairs. More specifically, they made more mistakes on the ‘high conflict’ pairs, with pairings that were more similar and thus more difficult to distinguish.

The amnesic effect of oxytocin in individuals who had experienced higher levels of emotional maltreatment found here is consistent with studies reporting decreased familiarity with adult faces (Herzmann et al, 2012) or decreased recall of reproduction related words (Heinrichs et al, 2004) after intranasal oxytocin administration. Moreover, similar to an earlier study (Heinrichs et al, 2004), we found that the amnesic effect of oxytocin was present for the implicit trials, where the participants had to derive the information about the presented pair, whereas the training itself did not reveal differences between the oxytocin and placebo condition. Our findings also suggest that the effects of oxytocin may differ depending on the uncertainty of the information being processed. More difficult pairs, with more similar temperamental characteristics (e.g., an infant being happy in 40% of the trials coupled with an infant being happy in 30% of the trials) yielded more errors after oxytocin administration in individuals who had experienced more emotional maltreatment.

Our results contrast with the studies showing memory enhancing effects of oxytocin (Guastella et al, 2008; Savaskan et al, 2008). Various sampling and methodological differences might be responsible for these differential effects of oxytocin. For example, the effects of oxytocin may be moderated by individual factors such as genetic background,
attachment style or early life experiences (Bakermans-Kranenburg et al., 2012; Bartz et al., 2010; Marsh et al., 2012). In the present study, we showed the importance of participants’ experiences of emotional maltreatment in moderating the effects of oxytocin. Participants with more emotional maltreatment experiences made more incorrect choices in differentiating between happy and sad infants after oxytocin administration. It should be noted that this outcome might depend on the distribution of maltreatment in the current sample. In clinical samples the amnesic effect of oxytocin might be even stronger.

Another issue is the difference in timing of oxytocin administration. In the present study, oxytocin was administered before the learning phase. The testing phase followed immediately after learning and therefore oxytocin levels were elevated throughout the session. In most of the earlier studies, the administration of oxytocin took place before or after the acquisition phase (Guastella et al., 2008; Savaskan et al., 2008), but the retrieval or testing phase in these studies was 24 hours after the administration of either oxytocin or placebo (Guastella et al., 2008; Rimmele et al., 2009). Intranasal oxytocin elevates salivary oxytocin levels substantially for at least seven hours after nasal spray administration (Van IJzendoorn et al., 2012b) but data about its effectiveness after 24 hours is lacking. Moreover, none of these study designs could assess the differences caused by oxytocin in the acquisition phase. In the present study, participants in the oxytocin and placebo condition with lower or higher experiences of emotional maltreatment did not differ in their learning to discriminate the happy and sad infants. Our results are in line with animal literature showing that oxytocin does not impact on learning but only on specific stages of memory processing (Kovács and Telegdy, 1982).

The type of stimuli also varies between studies. Whereas most of the earlier studies used adult faces or language memory, our study used infant faces coupled with infant crying and laughter sounds (tapping into attachment related memory processes). As infant cues differ from adult cues in how they are processed in the adult brain (Kringelbach et al., 2008), and as oxytocin selectively increases the preference for infants compared to adults (Marsh et al., 2012), it is possible that oxytocin administration acts differently in memory for infant stimuli. Moreover, childhood experiences might affect the salience and psychobiological relevance of emotional stimuli (Van Harmelen et al., 2012), thereby influencing infant emotion related memory processes as well.

Intranasal administration is the best available tool for testing the effects of oxytocin in humans experimentally. However, this method does not allow brain area specific delivery of the administered drug. Brain area specific oxytocin effects on memory
processes have been documented in animal literature. For example, while microinjection of oxytocin in limbic brain regions, dentate gyrus, or dorsal raphe nucleus attenuate memory consolidation; memory consolidation seems facilitated upon a microinjection into the dorsal septal nucleus (Kovács et al., 1979). Similarly varying processes occurring in different parts of the human brain after oxytocin administration might explain the inconsistencies among studies assessing effects of oxytocin on memory. In humans, the amygdala is implicated in partially mediating the effects of oxytocin (Kirsch et al., 2005; Riem et al., 2011). Differences in the bioavailability of oxytocin in the amygdala after intranasal administration may result in differential effects.

The mechanism behind the interaction between oxytocin and adverse childhood experiences affecting memory is not clear. One possibility is that these experiences impact upon amygdala activation. While emotional stimuli elicit increased amygdala activity in individuals who experienced emotional maltreatment (Van Harmelen et al., 2012), oxytocin decreases amygdala activity (Kirsch et al., 2005; Riem et al., 2011). Decreased amygdala activity may lower the salience of emotional cues with reduced memory of the cues as a result. Another possibility is that intranasal administration of oxytocin decreases cortisol responses to stressful tasks (Ditzen et al., 2009; Linnen et al., 2012), whereas experiences of emotional maltreatment are related to increased stress reactivity and higher cortisol levels (Carpenter et al., 2009). The interaction of oxytocin and maltreatment might alter cortisol reactivity to emotional stimuli, leading to impaired rather than facilitated memory processes (Smeets et al., 2008).

Oxytocin has long been acclaimed as a sociability hormone, increasing parental sensitivity (Naber et al., 2013) and interpersonal trust (De Dreu et al., 2010; Kosfeld et al., 2005). In decision making paradigms, participants make more prosocial choices towards in-group members after oxytocin administration (De Dreu et al., 2012). In the current study, participants with more experiences of maltreatment, who might be more at risk of abusing their own offspring (Sroufe, 2005), had more difficulty in identifying the happier infants after sniffing oxytocin. We might speculate that there is an adaptive evolutionary significance as in these participants oxytocin decreased aversion towards the sad infants and thus hampered distinguishing a happy infant from a sad infant. This lack of preference for the happier infants in the maltreated group might be seen as a protective mechanism enhancing their motivation to give the sad infants ‘another chance’.
Supplementary information (S1)

Participants’ self-rated mood

The PANAS was used to assess participants’ mood at baseline (T1) and post nasal spray administration (T2). The PANAS consists of 20 items, ten items for positive affect and ten for negative affect. Each item was rated on a 5-point rating scale ranging from not at all to a lot. Two scales, ‘positive affect’ (α (T1) = .80, α (T2) = .81) and ‘negative affect’ (α (T1) = .79, α (T2) = .57) were constructed by averaging the items.

Two 2x2x2 repeated measures mixed-design ANCOVAs were conducted to test the effect of nasal spray and time on participants’ mood (separately for positive affect and negative affect). Time (baseline T1 and post nasal spray T2) was the within-subject factor and nasal spray and emotional maltreatment were the between-subjects factors. Contraceptive use and basal salivary oxytocin levels were the covariates.

There was a significant time effect on participants’ rated positive affect, $F(1, 86) = 4.53, p = .036, \eta^2 = .050$, indicating that positive mood of the participants in all groups slightly declined over time. No other main or interaction effects were significant. For negative affect, we found a significant interaction between time and nasal spray, $F(1, 86) = 5.67; p = .019, \eta^2 = .062$, with a steeper decrease in negative affect in the oxytocin group compared to the placebo group (Figure S1). There were no main effects or interaction effects of emotional maltreatment group.

![Figure S1. Effect of oxytocin on the negative affect of the participants before (T1) and after nasal spray (T2). Participants in oxytocin group have a steeper decrease in negative affect ratings compared to placebo group. Data are plotted as mean ± SEM. * p < .05](image)
Supplementary information (S2)

Effect of oxytocin on perceived cuteness and temperament

Parental permission was obtained for the use of infant facial stimuli for research purposes, and was also approved by the Oxford Research Ethics Committee. At the beginning of the session (baseline; T1) and 45 minutes post nasal spray (T2), the participants rated the six infant faces with neutral facial expression on physiognomic and temperamental dimensions described by nine adjectives (attractive, cute, difficult, easy, irritable, responsive, secure, smart and spoilt). Face images were presented at the center of the screen with a vertical visual analogue scale (VAS) immediately to the right. The adjectives appeared at the ends of the VAS (for e.g. ‘cute’ at the top and ‘not cute’ at the bottom end of VAS). The rating bar started at the midpoint on the scale and participants could adjust the height of this bar using the ‘up’ and ‘down’ arrow keys on a standard keyboard. Scale scores on the VAS ranged from a maximum of 4 to a minimum of -4, with intervals of .0025. All nine adjectives for each infant were rated subsequently and the orders of both the adjectives and infant faces were randomized among participants.

Factor analysis was performed, with varimax rotation, on the nine characteristic ratings for the six infants separately. ‘Attractive’ and ‘cute’ ratings loaded on the same factor for all of the infants. ‘Difficult’, ‘easy’ (reversed) and ‘irritable’ ratings loaded on a factor for four out of six infants. Therefore, a ‘cuteness’ scale was created by averaging the ‘attractive’ and ‘cute’ ratings for each infant, for baseline ratings (T1) and for post nasal spray ratings (T2). Similarly, a ‘perceived temperament’ scale was created by averaging the ‘difficult’, ‘easy’ (reversed) and ‘irritable’ ratings for each infant.

Two repeated measures mixed-design ANCOVAs were performed to investigate the effects of nasal spray and emotional maltreatment on ratings of infant cuteness and perceived temperament. Within-subjects factors were time (baseline T1 and post nasal spray T2) and infant (6 infant faces). Nasal spray (oxytocin or placebo) and emotional maltreatment (higher or lower) were entered as between-subjects factors. Contraceptive use and basal salivary oxytocin levels were entered as covariates.

For the cuteness scale, there was a main effect of infant, $F(4.26, 366.19) = 3.57, p = .006, \eta^2 = .040$. Some infants were rated as significantly cuter than others. There was also a main effect of time, $F(1, 86) = 4.57, p = .035, \eta^2 = .050$, which was qualified by the significant time by nasal spray interaction, $F(1, 86) = 6.71, p = .011, \eta^2 = .072$. All infants
increased in mean cuteness ratings from T1 to T2, but there was a steeper increase in the ratings for the placebo group as compared to the oxytocin group (Figure S2). All other main and interactions effects were non-significant. There were no significant main or interaction effects for experienced emotional maltreatment.

Figure S2. Effects of oxytocin administration on cuteness scale of six infant faces before (T1) and after nasal spray (T2). Compared to placebo, the increase in cuteness ratings with time is lower in the oxytocin group. Data are plotted as mean ± SEM. *p < .05

For the temperament scale, there was a significant main effect of infant, $F(3.89, 334.45) = 2.99, p = .020, \eta^2 = .034$. Some infants were rated as having a more difficult temperament than others. No other main or interaction effects were significant (all $p > .050$). Participants in the oxytocin group did not differ from those in the placebo group in their perception of infant temperament. Experience of emotional maltreatment did not moderate participants’ perception of infant temperament.

Supplementary information (S3)

Statistical analyses

Analyses were performed using the IBM SPSS statistics 19 package. A series of analyses of co-variances (ANCOVAs) were performed to analyze the effects of oxytocin administration and moderation by self-reported experiences of childhood emotional maltreatment on accuracy of performance and ratings of infant characteristics. Nasal spray condition and the dichotomized emotional maltreatment scale were used as the predictor and moderator, respectively.

Covariates. Basal oxytocin levels were co-varied to control for individual differences in the oxytonergic system. Moreover, physiological oxytocin levels have been shown to be affected by stressful life experiences such as emotional abuse and neglect.
(Heim et al, 2008; Pierrehumbert et al, 2010). We therefore covaried basal oxytocin levels to control for any observed or unobserved individual differences in the oxytonergic system. One individual had extremely high basal oxytocin levels (> 3 standard deviations above the mean). The oxytocin level values for this individual were substituted with the next highest score (winsorized; Tabachnick and Fidell, 2006). Use of oral contraceptives was included as a covariate because there were significantly more participants using contraceptives in the oxytocin group than in the placebo group. This might result in a confounding effect of the use of contraceptives (and the resulting endocrinal and hormonal changes) on the memory related processes being tested here. Participants’ self-reported negative affect also differed across oxytocin and placebo groups. Participants in the oxytocin group had a steeper decrease in negative affect ratings from T1 to T2 as compared to placebo group (supplementary information S2). Difference scores for negative affect were therefore calculated by subtracting T1 ratings from T2 ratings and these difference scores were used as covariates in the learning and testing analyses. Participant’s mood might affect their performance at a task, as a more negative mood might lead to more negative perceptions of infant cues and therefore affect the memory in unknown ways. Given the differences in participant’s mood after oxytocin or placebo administration, individual participant mood was co-varied to control for any indirect effects of oxytocin on memory which are mediated by changes in mood. There was a significant time by nasal spray interaction effect for the infant cuteness ratings. All infants increased in mean cuteness ratings from T1 to T2, but there was a steeper increase in the ratings for the placebo group as compared to the oxytocin group (supplementary information S3). Difference scores for cuteness were therefore calculated by subtracting the average ratings of the six infants at T1 from the average ratings of the six infants at T2, and these difference scores were used as a covariate in the learning and testing analyses. As participants’ perception of the cuteness of an infant might also change the salience of the stimuli and thereby affect the familiarity of the face, we controlled for the change in attractiveness ratings of the faces, which were differentially affected by the nasal spray. Lastly, final level of learning was co-varied in the testing analyses in order to control for individual differences in performance during the training phase. This would result in differentiating the variability explained by the learning and would give clearer estimate of the effects of oxytocin and emotional maltreatment on memory for infant cues.

Greenhouse-Geisser corrections were performed wherever necessary. Post-hoc analyses were performed to localize significant differences between the four groups; placebo – lower levels of emotional maltreatment, placebo – higher levels of emotional mal-
treatment, oxytocin - lower levels of emotional maltreatment and oxytocin - higher levels of emotional maltreatment.

**Expectation effect.** At the end of the session, participants were asked which nasal spray they thought was administered to them (‘oxytocin’, ‘placebo’ or ‘don’t know’). As performance might be influenced by participants’ perception about the spray that was administered to them, we asked participants to guess the nasal spray that they received. Seventy percent of the participants reported not knowing which nasal spray they received. The remaining 30% of the participants were no more accurate than chance level in guessing their group correctly: 53% guessed incorrectly and 47% guessed correctly. We concluded that perception of the nasal spray did not affect the performance of participants.

**Supplementary information (S4)**

**Infant specific sub-pairs**

The derived pairs were classified into six infant specific sub-pairs, depending on the infant involved in it (for e.g. all the trials with 80% happy infant), in order to see if the effect of nasal spray was specific to some of the infants depending on their manipulated temperament. A MANCOVA was conducted with the infant specific pairs (six pairs) as dependent variables and nasal spray and emotional maltreatment as fixed factors and with the five covariates used in the overall correct analysis. There was a significant main effect of nasal spray on the 40% happy infant, \( F(1, 83) = 4.68, p = .033, \eta^2 = .053 \), qualified by a significant interaction between nasal spray and emotional maltreatment, \( F(1, 83) = 4.32, p = .041, \eta^2 = .049 \). A similar significant interaction was found for 60% happy infant, \( F(1, 83) = 4.47, p = .038, \eta^2 = .051 \). Post hoc analyses with the 40% happy infant showed that participants in the oxytocin group with higher maltreatment scores differed significantly from participants in the placebo group with higher maltreatment scores \( (p = .002) \). Participants in the oxytocin group with higher maltreatment scores had fewer correct responses \( (M = 0.60, SD = 0.03) \) than the participants in the placebo group with higher maltreatment scores \( (M = 0.75, SD = 0.04) \). For the 60% happy infant, participants in the oxytocin group with higher maltreatment scores differed significantly from participants in the placebo group with higher maltreatment scores \( (p = .015) \) and participants in the oxytocin group with lower maltreatment scores \( (p = .027) \). Participants in the oxytocin group with higher maltreatment scores had fewer correct responses \( (M = 0.60, SD = 0.03) \) than the participants in the placebo group with higher maltreatment scores \( (M = 0.72, SD = 0.03) \) and \( (M = 0.71, SD = 0.03) \) for participants in the oxytocin group with lower mal-
treatment scores (figure S3). There was no significant difference between the nasal spray groups with lower or higher maltreatment on their responses to the other infant pairs.

Figure S3. Correct responses in the testing phase with infant specific pairing. Participants with higher emotional maltreatment scores with oxytocin administration make significantly more incorrect choices as compared to higher emotional maltreatment scores with placebo administration for 60% happy infant and 40% happy infant and lower emotional maltreatment scores with oxytocin administration for 60% happy infant. The scores could range from 0 to 1. Data are plotted as mean ± SEM. * p < .05
References


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