ARCHIVAL REPORT

Oxytocin Modulates Amygdala, Insula, and Inferior Frontal Gyrus Responses to Infant Crying: A Randomized Controlled Trial

Madelon M.E. Riem, Marian J. Bakermans-Kranenburg, Suzanne Pieper, Mattie Tops, Maarten A.S. Boksem, Robert R.J.M. Vermeiren, Marinus H. van IJzendoorn, and Serge A.R.B. Rombouts

Background: Oxytocin facilitates parental caregiving and mother–infant bonding and might be involved in responses to infant crying. Infant crying provides information about the physical status and mood of the infant and elicits parental proximity and caregiving. Oxytocin might modulate the activation of brain structures involved in the perception of cry sounds—specifically the insula, the amygdala, and the thalamocingulate circuit—and thereby affect responsiveness to infant crying.

Method: In a randomized controlled trial we investigated the influence of intranasally administered oxytocin on neural responses to infant crying with functional magnetic resonance imaging. Blood oxygenation level–dependent responses to infant crying were measured in 21 women who were administered oxytocin and 21 women who were administered a placebo.

Results: Induced oxytocin levels reduced, experimentally, activation in the amygdala and increased activation in the insula and inferior frontal gyrus pars triangularis.

Conclusions: Our findings suggest that oxytocin promotes responsiveness to infant crying by reducing activation in the neural circuitry for anxiety and aversion and increasing activation in regions involved in empathy.

Key Words: Amygdala, fMRI, infant crying and parenting, insula, oxytocin, RCT

Infant crying alerts parents to the needs of the infant and elicits parental proximity and caregiving (1,2). Because young infants are fully dependent on their parents, correct perception and evaluation of infant crying by caregivers is crucial for infant survival. Several brain structures are involved in cry perception, specifically the insula and the thalamocingulate circuit that are activated when listening to infant crying (3,4). Oxytocin is a neuropeptide that facilitates parental caregiving and mother–infant bonding in various species, including humans (5–7). Reflecting its important role in maternal behavior, oxytocin might sensitize caregivers to variations in cry signals by modulating neural circuits related to the perception of infant crying and thus enhance responsiveness of caregivers. In this study, we examine the effects of experimentally elevated levels of oxytocin on neural responses to infant crying. Because crying has been found to be one of the major triggers of child abuse and neglect (8), examining the mechanisms that are involved in reactions of adults to infant crying is crucial.

Animal studies have shown that oxytocin is involved in lactation, pregnancy, and the onset of maternal behavior (5,7). Recent research also suggests an important role of oxytocin in human caregiving. Higher maternal oxytocin levels across pregnancy predict higher quality of postpartum maternal behavior (6). In addition, intranasally administered oxytocin has been shown to stimulate a range of social behaviors, including empathy (9,10), mind-reading (11), trust (12), and in-group altruism (13). Carter (5) argued that oxytocin might stimulate sensitive parenting in humans and other mammals by promoting acceptance of the newborn through reduction of fear to novelty and through enhancing prosocial behavior. This is in line with the suggestion of Heinrichs and Domes that oxytocin plays an important role as an underlying neurobiological mechanism for the anxiolytic/stress-protective effects of positive social interaction (14). Individuals with the potentially more efficient variant (GG) of the oxytocinergic receptor gene (OXTRrs53576) show, on the genetic level, reduced levels of stress and increased levels of empathy (15) as well as more sensitive parental interactions with toddlers (16).

An important component of parental sensitive caregiving is responsiveness to infant crying behavior. Infant crying provides information about the physical health and mental state of the infant, and the intensity of distress can be derived from the acoustics of the cry sound (17,18). The association between oxytocin and increased parental sensitivity might be partly due to the influence of oxytocin on responses to infant crying (19). Oxytocin might facilitate responsiveness to infant crying by modulating brain structures involved in cry perception, such as the thalamocingulate circuit, the insula, amygdala, and superior temporal gyrus (3,20,21). The thalamus is considered important for mammalian mother–infant attachment behavior (22). The insula is involved in maternal bonding and an important region implicated in empathy and emotion understanding (23), and it has been suggested that oxytocin is involved in the neurochemical mechanism underlying emotional empathy (10,24). Bos *et al.* (4) showed that testosterone administration increased activation in the thalamus and the insula during exposure to infant crying sounds, possibly through the effect of testosterone on the oxytocinergic system. Furthermore, vaginal delivery leads to increased oxytocin levels after vaginal-cervical stimulation, and mothers who experienced childbirth by vaginal delivery showed more brain activation in the superior temporal gyrus, the insula, and

From the Centre for Child and Family Studies (MMER, MJB-K, SP, MT, MHvIJ); Leiden Institute for Brain and Cognition (MMER, MJB-K, MT, MHvIJ, SARBR); Institute of Psychology (SARBR); Department of Child and Adolescent Psychiatry Curium (RRJMV); Department of Radiology (SARBR), Leiden University Medical Center, Leiden University, Leiden; Experimental Psychology (MT), University of Groningen, Groningen; Donders Institute for Brain, Cognition and Behavior (MASB), Radboud University, Nijmegen; Rotterdam School of Management (MASB), Erasmus University, Rotterdam, the Netherlands.

Address correspondence to Marian J. Bakermans-Kranenburg, Ph.D., Centre for Child and Family Studies, Leiden University, PO Box 9555, 2300 RB Leiden, the Netherlands; E-mail: bakermans@fsw.leidenuniv.nl.

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the anterior cingulate gyrus compared with mothers who had a Caesarean section delivery (25).

Although oxytocin seems to increase activation in brain regions involved in empathy (24), it decreases activation in the neural circuitry for anxiety and disgust (26,27). Kirsch *et al.* (28) found that oxytocin reduced amygdala activation during the perception of fear-inducing visual stimuli in men. This finding fits well with previously reported anxiolytic effects of oxytocin (29,30). Mothers experience strong emotional reactions to infant crying, ranging from empathy to anxiety and anger (31,32). Aversion or other negative emotional reactions might lead to insensitive parenting behaviors such as withdrawal or hostility to stop (having to listen to) infant crying (33); and high-pitched infant-crying in particular is an important trigger for child abuse (32,34). Because oxytocin decreases activation in the neural circuitry for anxiety and aversion, it might prevent parents from becoming over-reactive to the disturbing infant cry sounds. Although several studies have shown that oxytocin decreases amygdala reactivity to fearful stimuli in men, only one study has been conducted on the effects of oxytocin administration in women. Domes *et al.* (35) found that in women intranasal oxytocin increased amygdala activation in response to angry and happy adult faces, which is in contrast to reported effects in men (28). However, oxytocin might reduce amygdala activation in women listening to infant crying, which would be in line with the stress-reducing effects of endogenous oxytocin release in breastfeeding women (36,37).

To our knowledge this is the first randomized controlled study to examine the association between oxytocin manipulation and neural responses to infant crying. We studied the influence of intranasally administered oxytocin on neural responses to infant crying in women. We focused on neural responses to infant crying at different frequencies, because infant cries range from 500 Hz in normal, healthy infants to 900 Hz (and even higher) in infants in pain or with medical and neurological conditions (34,38,39). Blood oxygenation level–dependent response to infant crying was measured with functional magnetic resonance imaging (fMRI). Whole brain analysis was performed to explore the neural effects of oxytocin. We expected, in particular, that oxytocin administration would be related to increased activity in the thalamocingulate circuit and in the insula and decreased activity in the amygdala. Region of interest (ROI) analyses were conducted to examine the effects of oxytocin in these regions.

Methods and Materials

Participants

Participants were selected from a larger study investigating caregiving responses and physiological reactivity to infant crying (40). The original sample consisted of 50 male and 134 female adult twin pairs. Zygosity was determined on the basis of a zygosity questionnaire (41) and additional genetic analysis of selected polymorphisms. A group of 43 right-handed women were recruited, 21 from monozygotic (MZ) twin pairs and 22 from dizygotic (DZ) twin pairs, without children of their own; in good health; without hearing impairments, MRI contraindications, pregnancy, or psychiatric or neurological disorders; and screened for alcohol and drug use. One DZ sibling was excluded from the analyses, due to excessive head movement during fMRI scanning (peak displacement = 10 mm). Twin siblings of 10 participants did not participate, due to MRI contraindications or other exclusion criteria, resulting in a sample of 32 participants from twin pairs (9 MZ, 7 DZ) and 10 participants without twin sibling (3 MZ, 7 DZ). The mean age of the participants was 29.07 years (SD = 7.56, range 22–49). Sixty-nine percent of the participants used oral contraceptives. Permission for this study was obtained from the Medical Ethics Committee of the Leiden University Medical Center, and all participants gave informed consent.

Procedure

Participants were invited preferably in the luteal phase of their menstrual cycle. Approximately 36 min before the start of the fMRI data acquisition (M = 35.86, SD = 5.12) subjects took nasal spray containing oxytocin or placebo. Time between oxytocin/placebo administration and data acquisition was similar to previous fMRI studies (42,43). Participants were instructed to comfortably position themselves on the scanner bed. Cushions were placed between the head coil and the participant to prevent head movement. Participants were instructed to attend to the sounds they would hear. Before drug administration and after fMRI scanning, participants completed a mood questionnaire to track mood changes after drug administration. Participants rated on 7-point Likert scales how much anxiety, anger, frustration, empathy, happiness, and calmness they felt (28). In addition, they rated the perceived urgency of the infant cry sounds on a 5-point Likert scale after fMRI scanning (44).

Cry Paradigm

Cry sounds were derived from the spontaneous crying of a healthy 2-day old infant. A 10-sec portion of the sustained period of crying was selected. The peak fundamental frequencies (Peak F0) of the entire cry were 515 ± 15 Hz. Two new 10-sec cry sounds with overall Peak F0 of 714.5 Hz (700 Hz cry) and 895.8 Hz (900 Hz cry) were created by digitally increasing the pitch of the original cry (32,45–47). Neutral auditory control stimuli were created identical to the original auditory stimuli in terms of duration, intensity, spectral content, and amplitude envelope but lacking an emotional meaning. Cry and control sounds were presented in eight cycles, each cycle consisting of six sounds (Cry 500 Hz, Cry 700 Hz, Cry 900 Hz, Control 500 Hz, Control 700 Hz, Control 900 Hz). The order of presentation of sounds within each cycle was random; the intertrial interval was 6 sec (Figure 1).

Oxytocin Versus Placebo

One sibling from each twin pair was randomly assigned to the oxytocin condition, and the other sibling was assigned to the placebo condition. Participants without a twin sibling were also randomly assigned to the oxytocin or placebo condition. Approximately 36 min before the start of the fMRI data acquisition, subjects took six puffs of nasal spray containing 4 IU/puff of oxytocin (24 IU total, RVG Number 03716, Sandoz b.v.) or six puffs of a placebo-spray (sodium chloride solution) under supervision of the experimenter. Drug administration was double-blind.

Image Acquisition

Scanning was performed with a standard whole-head coil on a 3-T Philips Achieva MRI system (Philips Medical Systems, Best, the Netherlands) in the Leiden University Medical Center. First, a T1-weighted anatomical scan was acquired (flip angle = 8°, 140 slices, voxel size .875 × .875 × 1.2 mm). For fMRI, a total of 360 T2*-weighted whole-brain echoplanar images were acquired (repeti-

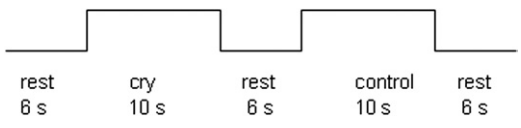

Figure 1. The cry paradigm. Cry sounds and control sounds were presented for 10 sec, followed by a rest period of 6 sec.

tion time = 2.2 sec; echo time = 30 msec, flip angle = 80°, 38 transverse slices, voxel size 2.75 × 2.75 × 2.75 mm [+ 10% interslice gap]). Participants listened to the sounds through MRI-compatible headphones. In accordance with Leiden University Medical Center policy, all anatomical scans were examined by a radiologist from the Radiology Department. No anomalous findings were reported.

fMRI Data Analysis

Data analysis was carried out with fMRI Expert Analysis Tool Version 5.98, part of FSL (FMRIB's Software Library, http://www.FMRIb.ox.ac.uk/fsl; 48). The following prestatistics processing was applied: motion correction (49), non-brain removal (50), spatial smoothing with a Gaussian kernel of full-width-at-half-maximum 5.0 mm, and high-pass temporal filtering (high-pass filter cutoff = 50.0 sec). Functional scans were registered to T1-weighted images, which were registered to standard space (49,51).

In native space, functional activation was examined with general linear model analysis. Each sound (Cry 500 Hz, 700 Hz, 900 Hz, and Control 500 Hz, 700 Hz, 900 Hz) was modeled separately as a square-wave function. Each predictor was then convolved with a double γ hemodynamic response function, and its temporal derivative was added to the model, giving 12 predictors. To identify regions involved in the perception of infant crying, the following contrasts were assessed: 1) $Cry_{combined500,700,900Hz} > Control_{combined500,700,900Hz}$ 2) $Cry_{500Hz} > Control_{500Hz}$ 3) $Cry_{700Hz} > Control_{700Hz}$ 4) $Cry_{900Hz} > Control_{900Hz}$. These first-level contrast images and the corresponding variance images were transformed to standard space and submitted to second-level mixed-effects group whole brain analyses. Group means were tested with one-sample *t* tests, and we tested for group differences with two-sample *t* tests on these contrasts with the oxytocin versus placebo group comparison ($Oxytocin^{Cry>Control} > Placebo^{Cry>Control}$). We also examined, in a similar fashion to that by Domes *et al.* (35), reduced brain activation in the oxytocin group compared with the placebo group in the reverse contrast ($Oxytocin^{Cry>Control} < Placebo^{Cry>Control}$). The statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$ (52).

Additionally, ROI analyses were performed with the FSL software to investigate changes in activation of a priori specified regions that were related to the perception of infant crying in the literature. These regions are the amygdala, thalamus, and insula (3,4,53) and were defined with the Harvard–Oxford cortical atlas (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). The ROI analyses were limited to these search regions, applying the same statistical threshold as for the whole brain analyses, although correcting only for the size of ROI volumes.

We included menstrual cycle and use of oral contraceptives in the whole brain and ROI analyses as confound regressors in the model. To study the possible effect of various a priori defined parameters on the observed changes in brain activation, we also computed mean parameter estimates of the contrasts that yielded significant activation with Featquery (http://www.FMRIb.ox.ac.uk/fsl/feat5/featquery.html). This was done for each activated region. Univariate analyses of covariance were performed with the mean parameter estimate as dependent variable, oxytocin/placebo as between-subjects factor, and age and time between oxytocin/placebo administration and fMRI data acquisition as covariates. In addition, we performed univariate analyses of variance with the mean parameter estimate as dependent variable; oxytocin/placebo as between-subjects factor; and menstrual cycle (follicular or luteal phase), use of oral contraceptives, and MZ twin/DZ twin/participant without twin sibling (sibling group) as between-subjects factors to examine the influence of potential confounding variables. Mean *Z* values for regions with a change in brain activation after oxytocin administration were calculated with Featquery for visualization purposes.

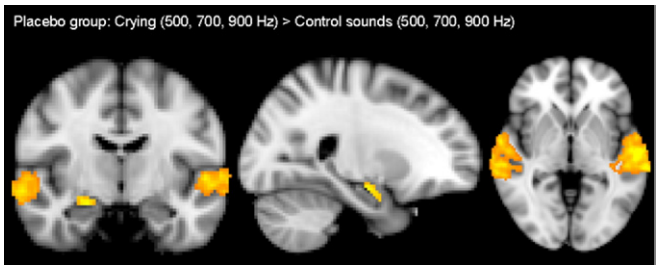

Figure 2. Significant activation in the bilateral superior and middle temporal gyrus and the amygdala for the contrast Crying (500, 700, 900 Hz) > Control sounds (500, 700, 900 Hz). Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The amygdala activation was revealed by region of interest (ROI) analysis limited to this search region, applying the same statistical threshold but correcting only for the size of the ROI volume.

Results

Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. In addition, we included use of oral contraceptives and menstrual cycle as confound regressors in the model. The main contrast of infant crying (combined 500 Hz, 700 Hz, 900 Hz) versus control sounds (combined 500 Hz, 700 Hz, 900 Hz) revealed stronger bilateral activation in the superior and middle temporal gyrus in the placebo condition (Figure 2, Table 1). The ROI analyses were conducted to examine whether there was activation in a priori defined areas of interest (insula, amygdala, and thalamus). We found stronger activation in the right amygdala when participants in the placebo group listened to infant crying compared with the control sounds. The thalamus and insula did not show a significant difference in activation during infant crying compared with control sounds.

Similar to the main comparison of total infant crying with control sounds, the contrast of the 500 Hz cry sound versus the 500 Hz control sound (contrast 2) revealed significant activation in the bilateral superior temporal gyrus and the left occipital fusiform gyrus in the placebo condition (Table 1). The ROI analyses did not show significant activation in the thalamus or insula. However, there was significant activation in the right amygdala. In the placebo condition we also found stronger activation in the bilateral superior temporal gyrus during the perception of the 700 Hz cry sound compared with the 700 Hz control sound (contrast 3). The location of the peak voxel of the cluster in the right hemisphere was located in the middle temporal gyrus. Again, this contrast revealed significant activation in the right amygdala but not in the insula or thalamus. Lastly, no significantly different activation was observed during the perception of the 900 Hz cry sound compared with the 900 Hz control sound in the whole brain analysis (contrast 4). The ROI analyses revealed significant activation in the right amygdala but no significant (de-)activation in the insula or thalamus.

To examine whether oxytocin affected neural responses to crying (combined 500 Hz, 700 Hz, 900 Hz), we contrasted the oxytocin group versus the placebo group ($Oxytocin^{Cry>Control} > Placebo^{Cry>Control}$ and $Oxytocin^{Cry>Control} < Placebo^{Cry>Control}$). These contrasts did not yield significantly different activation in the whole brain analysis. However, ROI analysis showed that, compared

Table 1. MNI Coordinates, Cluster Size, and Z-Max Values for Significantly Activated Clusters

Experimental Effect Region	MNI Coordinates x	 y	 z	Cluster Size	Peak *Z*
Main effects in placebo group					
Cry > Control					
L superior temporal gyrus	−62	−14	0	2314	5.62
R middle temporal gyrus	62	−26	−4	2367	4.76
R amygdala	26	−12	−14	136	4.02[a]
Cry 500 Hz > Control 500 Hz					
L superior temporal gyrus	−58	−20	0	761	4.94
R superior temporal gyrus	62	−22	−4	611	3.97
L occipital fusiform gyrus	−30	−76	−12	520	3.47
R amygdala	22	−10	−16	27	3.42[a]
Cry 700 Hz > Control 700 Hz					
L superior temporal gyrus	−62	−16	2	1797	4.75
R middle temporal gyrus	72	−34	2	853	3.78
R amygdala	20	−8	−16	59	2.95[a]
Cry 900 Hz > Control 900 Hz					
R amygdala	20	−2	−20	35	3.25[a]
Sounds × Drug Effects					
$OXT^{Cry > Control} < PLA^{Cry > Control}$					
R amygdala	26	−12	−14	58	3.28[a]
$OXT^{Cry\ 500\ Hz > Control\ 500\ Hz} >$ $PLA^{Cry\ 500\ Hz > Control\ 500\ Hz}$					
R planum polare	−52	−8	0	780	3.93
L inferior frontal gyrus	50	16	−4	747	3.97

$P < .05$, corrected by whole brain cluster threshold ($z > 2.3$); use of oral contraceptives and menstrual cycle included as confound regressors in the model. MNI, Montreal Neurological Institute; OXT, oxytocin; PLA, placebo.

L, left; MNI, Montreal Neurological Institute; OXT, oxytocin; PLA, placebo; R, right.

[a]Region of interest analysis, $p < .05$, corrected by cluster threshold ($z > 2.3$).

with the placebo group, participants who received oxytocin showed reduced activation in the right amygdala when they listened to infant crying (top panel in Figure 3, Table 1). This contrast did not reveal significant activation in the other ROIs.

We also examined the effect of oxytocin in the 500 Hz condition ($Oxytocin^{Cry500Hz>Control500Hz} > Placebo^{Cry500Hz>Control500Hz}$). Whole brain analysis indicated that oxytocin increased bilateral insula and inferior frontal gyrus pars triangularis activation (Harvard–Oxford cortical atlas: frontal operculum) (lower panel in Figure 3, Table 1). The cluster in the right hemisphere extended more laterally, and the location of the peak voxel was in the planum polare. The ROI analysis of responses to 500 Hz cry sounds showed that oxytocin increased left insula activation, but there was no significant effect in the thalamus or amygdala. Whole brain and ROI analyses did not show significant effects of oxytocin on neural responses to infant crying at 700 Hz and 900 Hz.

The univariate analyses of (co)variance with age, time between placebo/oxytocin administration and fMRI data acquisition, menstrual cycle, sibling group, and use of oral contraceptives showed that none was significantly associated with change in brain activation, either at whole group level (placebo and oxytocin) or for the difference between the oxytocin and placebo condition. Whole brain and ROI analyses were repeated after exclusion of four participants who were older than 41 years to examine whether advanced age influenced the effects of oxytocin (54). This did not result in different findings: oxytocin significantly increased inferior frontal gyrus and insula activation and significantly reduced right amygdala activation.

To control for nonspecific effects of oxytocin on self-reported mood and perceived urgency of the cry sounds, we conducted repeated-measures analyses of variance with group (oxytocin and placebo) as between-subject factor and time (time 1: before drug administration, and time 2: after scanning) as within-subject factor. There were no significant time × group interaction effects on any of the mood items: anger [$F(1,40) = .02$, $p = .89$], frustration [$F(1,40) = .25$, $p = .62$], anxiety [$F(1,40) = 3.07$, $p = .09$], empathy [$F(1,40) = .03$, $p = .86$], happiness [$F(1,40) = 1.08$, $p = .30$], and calmness [$F(1,40) = .40$, $p = .53$]. Moreover, there were no significant effects of oxytocin on perceived urgency of infant crying at 500 Hz [$F(1,39) = 1.58$, $p = .22$], 700 Hz [$F(1,39) = 2.00$, $p = .17$], or 900 Hz [$F(1,39) = 2.23$, $p = .14$].

Discussion

Our study demonstrates that oxytocin administration is related to reduced right amygdala activation and enhanced insula and inferior frontal gyrus activation when exposed to infant crying compared with control sounds. Several studies have shown that the amygdala is involved in the perception of infant stimuli (3,4,21) and that oxytocin reduces amygdala activation during the perception of fear-inducing social stimuli (28,55,56). Decreased amygdala activation might promote responsiveness to infant crying by preventing parents from being overwhelmed by anxious or aversive feelings. This fits well with findings of stress-reducing effects of oxytocin in lactating mothers (36,37).

Although oxytocin reduced neural activation in the amygdala, it increased activation in regions associated with empathy and mother–infant bonding, the insula and the inferior frontal gyrus pars triangularis. The increase in insula and inferior frontal gyrus activation only occurred during the 500 Hz crying condition, possibly because this unaltered cry sound was most naturalistic and characteristic for a normal healthy infant. Previous studies have shown that the insula is involved in the perception of the own infant's sad faces (53) and the inferior frontal gyrus is important for affective prosodic comprehension (57). Empathy is an important prerequisite of parental sensitivity, defined as the ability of parents to perceive child signals, to interpret these signals correctly, and to respond to them promptly and appropriately (58). Indeed, empathy has been found related to parental responsiveness to infant signals (59–61). It is based on a neural simulation mechanism that is activated both when subjected to an emotion and when observing someone else experiencing the emotion, thus enabling humans to understand the emotions of others (62,63). The insula and inferior frontal gyrus are suggested to play an important role in this simulation process (63–66), and oxytocin might facilitate responsiveness to infant crying by increasing activation in brain regions important for empathy. Our findings are in line with results from a study by Bos *et al.* (4), who found that testosterone increases insula activation in response to infant crying, possibly by influencing the oxytocinergic system. Testosterone has been linked to oxytocin as it is metabolized into estradiol, which subsequently influences oxytocin levels (67,68).

In the placebo group, we found an increased activation in the bilateral superior and middle temporal gyrus and the occipital fusiform gyrus when listening to infant crying compared with control sounds. These regions are important for language processing and social perception (57,69). Previous studies have also shown involvement of the superior temporal gyrus and the fusiform gyrus on neural responses to infant stimuli (21,53). In addition, we found

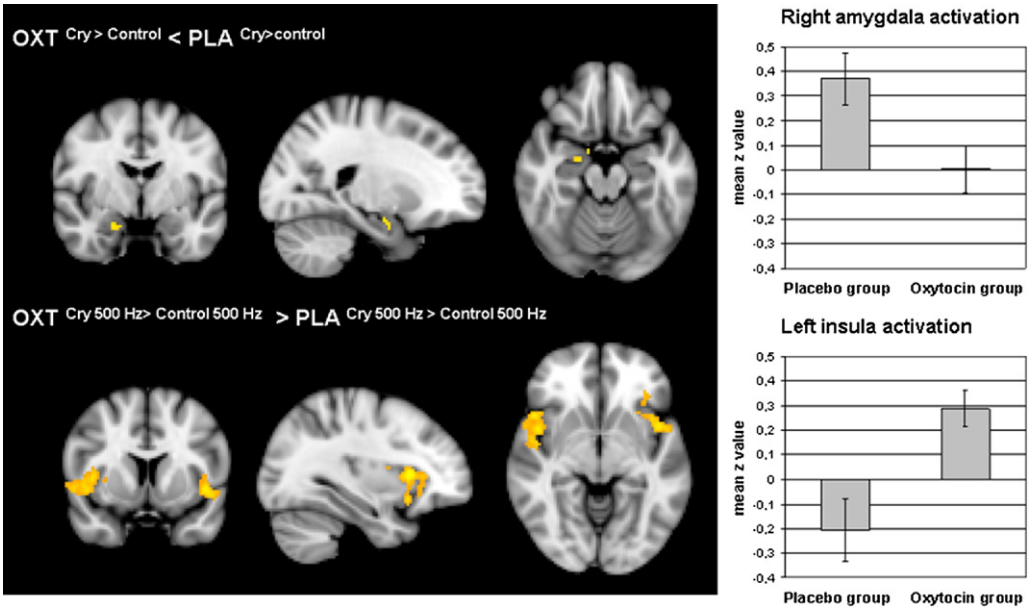

Figure 3. Top panel: oxytocin (OXT) effect on right amygdala activation and mean *Z* values and standard errors for the OXT and placebo (PLA) group for the contrast Cry combined > Control combined in the amygdala. Lower panel: OXT effect on bilateral insula and inferior frontal gyrus pars triangularis activation and mean *Z* values and standard errors for the OXT and PLA group for the contrast Cry 500 > Control 500 in the left insula. Both statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The amygdala activation was revealed by region of interest (ROI) analysis limited to this search region, applying the same statistical threshold but correcting only for the size of the ROI volume.

increased activation in the amygdala during infant crying compared with control sounds. Amygdala activation was restricted to the right hemisphere. This is in line with previous studies of responsiveness to infant crying (3,4,20). It has been suggested that this right hemisphere dominance has evolved, because most women carry their infant on their left arm (70–72).

The thalamus did not show significantly increased activation during exposure to infant crying, in contrast with our expectations, and no different activation in the oxytocin compared with the placebo group. It should be noted that previous findings are equivocal, with some studies reporting increased thalamic activation during the perception of infant crying (4,20,21) and other studies not finding significant thalamic activation to infant cues (3,53). These equivocal results might be explained by different characteristics of cry and control stimuli. For instance, in some studies, mothers listened to the crying of their own infant, whereas other studies used cry sounds of unknown infants. Swain *et al.* (21) showed that, among other regions, the thalamus was significantly more active when mothers listened to the cry of their own infant compared with cry sounds of unknown infants. Several animal studies have shown that the density of oxytocin receptors is high in the thalamus, in particular in social species (73,74), but the number of studies reporting oxytocin effects on thalamic activation is limited (75). More research is needed to clarify the role of the thalamus in the perception of infant crying and whether it is affected by oxytocin.

Age, menstrual cycle, sibling group, use of oral contraceptives, and time between placebo/oxytocin administration and fMRI data acquisition were not associated with (differences in) brain activation. Although several studies have shown that oxytocin levels fluctuate throughout the menstrual cycle (76–78), it is possible that the effect of intranasally administered oxytocin on neural activity in response to cry sounds was large enough to overrule the effects caused by normal fluctuations. Moreover, most of the participants used oral contraceptives, dampening menstrual cycle-related hormonal fluctuations (79). Furthermore, our findings of reduced amygdala activation and increased insula activation were not influenced by a general change in mood, because oxytocin did not affect self-reported emotional states. These results converge with several other studies that reported no effects of oxytocin on emotional state (28,80).

The limitations of our study should be acknowledged. First, the physiological effects induced by intranasally administered oxytocin are not well-understood and might be different from the effects of endogenous oxytocin secretion. Second, a between-subjects design implies the risk of pre-existing differences between the oxytocin and placebo group that might have influenced the results. However, most of our participants were MZ and DZ twin pairs, perfectly matched on age and global child-rearing experiences and even on genotype in MZ twin pairs. Third, we suggested that in decreasing amygdala activation oxytocin might inhibit aversive responses to infant crying. In a future study, the effects of oxytocin on behavioral responses to infant crying mediated by amygdala activation should be examined. Furthermore, oxytocin might increase stress responses when parents are confronted with threatening stimuli that could harm the infants (13). Domes *et al.* (35) found increased amygdala activation in women in the oxytocin condition in response to fearful or angry adult faces. This finding seems in contrast to the previously reported effects of oxytocin found in men, but it might reflect enhanced vigilance to threat signals evolved to protect the child, thus triggering an aggressive reaction to frightening faces of adult strangers (13). In an ideal future study, the Domes *et al.* (35) paradigm would be used together with the cry paradigm, preferably with matched male and female respondents. Lastly, our findings can only be generalized to women without children.

In conclusion, this is the first study to show the effect of oxytocin administration on neural responses to infant crying, extending previous findings indicating a central role for oxytocin in parental caregiving and attachment formation (80,81). Our findings suggest that oxytocin promotes responsiveness to infant crying by reducing activation in the neural circuitry for anxiety and aversion and by

increasing activation in regions involved in empathy. Infant crying might trigger child abuse or neglect (8). However, not all parents with irritable infants become abusive, and differences in oxytocin levels might explain why some parents remain sensitive whereas other parents lack the empathic ability to abstain from harsh or even abusive responses to their infant's crying.

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Supplementary material cited in this article is available online.

1. Bowlby J (1969/82): *Attachment and Loss, vol. 1.* New York: Basic Books.
2. Zeifman DM (2001): An ethological analysis of human infant crying: Answering Tinbergen's four questions. *Dev Psychobiol* 39:265–285.
3. Seifritz E, Esposito F, Neuhoff JG, Lüthi A, Mustovic H, Dammann G, *et al.* (2003): Differential sex-independent amygdala response to infant crying and laughing in parents versus nonparents. *Biol Psychiatry* 54:1367–1375.
4. Bos PA, Hermans EJ, Montoya ER, Montoya ER, Ramsey NF, Van Honk J (2010): Testosterone administration modulated neural responses to crying infants in young females. *Psychoneuroendocrinology* 35:114–121.
5. Carter CS (1998): Neuroendocrine perspectives on social attachment and love. *Psychoneuroendocrinology* 23:779–818.
6. Feldman R, Weller A, Zagoori-Sharon O, Levine A (2007): Evidence for a neuroendrocrinological foundation of human affiliation. *Psychol Sci* 18: 965–970.
7. Insel TR (2010): The challenge of translation in social neuroscience: A review of oxytocin, vasopressin, and affiliative behavior. *Neuron* 65: 768–779.
8. Reijneveld SA, Van der Wal MF, Brugman E, Sing RA, Verloove-Vanhorick SP (2004): Infant crying and child abuse. *Lancet* 364:1340–1342.
9. Bartz JA, Zaki J, Bolger N, Hollander E, Ludwig NN, Kolevzon A, Ochsner KN (2010): Oxytocin selectively improves empathic accuracy. *Psychol Sci* 21:1426–1428.
10. Hurlemanm R, Patin A, Onur OA, Cohen MX, Baumgarter T, Metzler S, *et al.* (2010): Oxytocin enhances amygdala-dependent, socially reinforced learning and emotional empathy in humans. *J Neurosci* 30:4999–5007.
11. Domes G, Heinrichs M, Michel A, Berger C, Herpertz SC (2007): Oxytocin improves "mind-reading" in humans. *Biol Psychiatry* 61:731–733.
12. Kosfeld M, Heinrichs M, Zak PJ, Fischbacher U, Fehr E (2005): Oxytocin increases trust in humans. *Nature* 435:673–676.
13. De Dreu CKW, Greer LL, Handgraaf MJJ, Shalvi S, Van Kleef GA, Baas M, *et al.* (2010): The neuropeptide oxytocin regulates parochial altruism in intergroup conflict among humans. *Science* 328:1408–1411.
14. Heinrichs M, Domes G (2008): Neuropeptides and social behavior: Effects of oxytocin and vasopressin in humans. *Prog Brain Res* 170:337–350.
15. Rodrigues SM, Saslow LR, Garcia N, John OP, Keltner D (2009): Oxytocin receptor genetic variation relates to empathy and stress reactivity in humans. *Proc Natl Acad Sci U S A* 106:21437–21441.
16. Bakermans-Kranenburg MJ, Van Ijzendoorn MH (2008): Oxytocin receptor OXTR and serotonin transporter 5-HTT genes associated with observed parenting. *Soc Cogn Affect Neurosci* 3:128–134.
17. Murray AD (1979): Infant crying as an elicitor of parental behavior: Examination of two models. *Psychol Bull* 86:191–215.
18. Gustafson GE, Wood RM, Green JA (2000): Can we hear the causes of infant crying? In: Barr RG, Hopkins B, Green JA, editors. *Crying as a Sign, a Symptom, and a Signal*. London: Mac Keith Press, 8–23.
19. Riem MME, Pieper S, Out D, Bakermans-Kranenburg MJ, Van IJzendoorn MH (in press) Oxytocin receptor gene and depressive symptoms associated with physiological reactivity to infant crying [published online ahead of print April 16]. *Soc Cogn Affect Neurosci.*
20. Lorberbaum JP, Newman JD, Horwitz AR, Dubno JR, Lydiard RB, Hamner RB, *et al.* (2002): A potential role for thalamocingulate circuitry in human maternal behavior. *Biol Psychiatry* 51:431–445.
21. Swain JE, Tasgin E, Mayes LC, Feldman R, Constable RT, Leckman JF (2008): Maternal brain response to own baby-cry is affected by Cesarean section delivery. *J Child Psychol Psychiatry* 49:1042–1052.
22. MacLean PD (1990): *The Triune Brain in Evolution: Role in Paleocerebral Functions*. New York: Plenum Press.
23. Bartels A, Zeki S (2004): The neural correlates of maternal and romantic love. *Neuroimage* 21:1155–1166.
24. Shamay-Tsoory SG (2011): The neural bases of empathy. *Neuroscientist* 17:18–24.
25. Swain JE, Tasgin E, Mayers LC, Feldman R, Constable RT, Leckman JF (2008): Maternal brain response to own baby-cry is affected by cesarean section delivery. *J Child Psychol Psychiatry* 49:1042–1052.
26. Gamer M, Zurowski B, Büchel C (2010): Different amygdala subregions mediate valence-related and attentional effects of oxytocin in humans. *Natl Acad Sci U S A* 107:9400–9405.
27. Stark R, Schienle A, Walter B, Kirsch P, Sammer G, Ott U, *et al.* (2003): Hemodynamic responses to fear and disgust-inducing pictures: An fMRI study. *Int J Psychophysiol* 57:61–67.
28. Kirsch P, Esslinger C, Chen Q, Mier D, Lis S, Siddhanti S, *et al.* (2005): Oxytocin modulates neural circuitry for social cognition and fear in humans. *J Neurosci* 25:11489–11493.
29. McCarthy MM, McDonald CH, Brooks PJ, Goldman D (1996): An anxiolytic action of oxytocin is enhanced by estrogen in the mouse. *Physiol Behav* 60:1209–1215.
30. Heinrichs M, Baumgartner T, Kirschbaum C, Ehlert U (2003): Social support and oxytocin interact to suppress cortisol and subjective responses to psychological stress. *Biol Psychiatry* 54:1389–1398.
31. Dix TH (1991): The affective organization of parenting: Adaptive and maladaptive processes. *Psychol Bull* 110:3–25.
32. Out D, Pieper S, Bakermans-Kranenburg MJ, Zeskind PS, Van Ijzendoorn MH (2010): Intended sensitive and harsh caregiving responses to infant crying: The role of cry pitch and perceived urgency in an adult twin sample. *Child Abus Negl* 34:863–873.
33. Dix T, Gershoff ET, Meunier MN, Miller PC (2004): The affective structure of supportive parenting: Depressive symptoms, immediate emotions, and child-oriented motivation. *Dev Psychol* 40:1212–1217.
34. Soltis J (2004): The signal functions of early infant crying. *Behav Brain Sci* 27:443–490.
35. Domes G, Lischke A, Berger C, Grossmann A, Hauenstein K, Heinrichs M, Herpertz SC (2010): Effects of intranasal oxytocin on emotional face processing in women. *Psychoneuroendocrinology* 35:83–93.
36. Heinrichs M, Meinlschmidt G, Neumann I, Wagner S, Kirschbaum C, Ehlert U, Hellhammer DH (2001): Effects of suckling on hypothalamic-pituitary-adrenal axis responses to psychosocial stress in postpartum lactating women. *J Clin Endocrinol Metab* 86:4798–4804.
37. Heinrichs M, Neumann I, Ehlert U (2002): Lactation and stress: Protective effects of breast-feeding in humans. *Stress* 5:195–203.
38. Porter FL, Porges SW, Marshall RE (1988): Newborn pain cries and vagal tone: Parallel changes in response to circumcision. *Child Dev* 59:495–505.
39. Zeskind PS, Collins V (1987): Pitch of infant crying and caregiving responses in a natural setting. *Infant Bev Dev* 10:501–504.
40. Out D, Pieper S, Bakermans-Kranenburg MJ, Van IJzendoorn MH (2010): Physiological reactivity to infant crying: A behavioral genetic study. *Genes Brain Behav* 9:868–876.
41. Magnus P, Berg K, Nance WE (1983): Predicting zygosity in Norwegian twin pairs born 1915–1960. *Clin Genet* 24:103–112.
42. Rimmele U, Hediger K, Heinrichs M, Klaver P (2009): Oxytocin makes a face in memory familiar. *J Neurosci* 29:38–42.
43. Marsh AA, Yu HH, Pine DS, Blair RJR (2010): Oxytocin improves specific recognition of positive facial expressions. *Psychopharmacology (Berl)* 209:225–232.
44. Zeskind PS, Marshall TR (1988): The relation between variations in pitch and maternal perception of infant crying. *Child Dev* 59:193–196.
45. Dessureau BK, Kurowski CO, Thompson NS (1998): A reassessment of the role of pitch and duration in adults' responses to infant crying. *Infant Behav Dev* 21:367–371.
46. Schuetze P, Zeskind PS (2001): Relations between women's depressive symptoms and perceptions of infant distress signals varying in pitch. *Infancy* 2:483–499.

47. Schuetze P, Zeskind PS, Eiden RD (2003): The perceptions of infant distress signals varying in pitch by cocaine-using mothers. *Infancy* 4:65–83.
48. Smith SM, Jenkinson M, Woolrich MW, Beckmann CF, Behrens TE, Johansen-Berg H, *et al.* (2004): Advances in functional and structural MR image analysis and implementation as FSL. *Neuroimage* 23(suppl 1):208–219.
49. Jenkinson M, Bannister P, Brady M, Smith S (2002): Improved optimization for the robust and accurate linear registration and motion correction of brain images. *Neuroimage* 17:825–841.
50. Smith SM (2002): Fast robust automated brain extraction. *Hum Brain Mapp* 17:143–155.
51. Jenkinson M, Smith S (2001): A global optimisation method for robust affine registration of brain images. *Med Image Anal* 5:143–156.
52. Worsley KJ (2001): Statistical analysis of activation images. In: Jezzard PM, Matthews PM, Smith SM, editors. *Functional MRI: An Introduction to Methods*. New York: Oxford University Press, 251–270.
53. Strathearn L, Fonagy P, Amico J, Montague PR (2009): Adult attachment predicts maternal brain and oxytocin response to infant cues. *Neuropsychopharmacology* 34:2655–2666.
54. Al-Azzawi F, Palacios S (2009): Hormonal changes during menopause. *Maturitas* 63:135–137.
55. LeDoux JE (2000): Emotion circuits in the brain. *Annu Rev Neurosci* 23: 155–184.
56. Petrovic P, Kalisch R, Singer T, Dolan RJ (2008): Oxytocin attenuates affective evaluations of conditioned faces and amygdala activity. *J Neurosci* 28:6607–6615.
57. Leitman DI, Wolf DH, Ragland JD, Laukka P, Loughead J, Valdez JN, *et al.* (2010): "It's not what you say, but how you say it": A reciprocal temporofrontal network for affective prosody. *Front Hum Neurosci* 4:1–13.
58. Ainsworth MDS, Blehar MC, Waters E, Wall S (1978): *Patterns of Attachment. A Psychological Study of the Strange Situation*. Hillsdale, New Jersey: Lawrence Erlbaum.
59. Gondoli DM, Silverberg SB (1997): Maternal emotional distress and diminished responsiveness: The mediating role of parenting efficacy and parental perspective taking. *Dev Psychol* 33:861–868.
60. Leerkes EM (2010): Predictors of maternal sensitivity to infant distress. *Parent Sci Pract* 10:219–239.
61. Feldman R (2007): On the origins of background emotions: From affect synchrony to symbolic expression. *Emotion* 7:601–611.
62. Iacoboni M (2009): Imitation, empathy and mirror neurons. *Annu Rev Psychol* 60:653–670.
63. Gallese V, Keysers C, Rizzolatti G (2004): A unifying view of the basis of social cognition. *Trends Cogn Sci* 8:396–403.
64. Chakrabarti B, Bullmore E, Baron-Cohen S (2006): Empathizing with basic emotions: Common and discrete neural substrates. *Soc Neurosci* 1:364–384.
65. Decety J, Jackson PL (2004): The functional architecture of human empathy. *Behav Cogn Neurosci Rev* 3:71–100.
66. Jabbi M, Swart M, Keysers C (2007): Empathy for positive and negative emotions in the gustatory cortex. *Neuroimage* 34:1744–1753.
67. Choleris E, Devidze N, Kavaliers M, Pfaff DW (2008): Steroidal/neuropeptide interactions in hypothalamus and amygdala related to social anxiety. *Prog Brain Res* 170:291–303.
68. Cornil CA, Ball GF, Balthazart J (2006): Functional significance of the rapid regulation of brain estrogen action: Where do the estrogens come from? *Brain Res* 1126:2–26.
69. Baron-Cohen S, Ring HA, Wheelwright S, Bullmore ET, Brammer MJ, Simmons A, Williams SC (1999): Social intelligence in the normal and autistic brain: An fMRI study. *Eur J Neurosci* 11:1891–1898.
70. Sieratzki JS, Woll B (1996): Why do mothers cradle babies on their left? *Lancet* 347:1746–1748.
71. Reissland N, Hopkins B, Helms P, Williams B (2009): Maternal stress and depression and the lateralization of infant cradling. *J Child Psychol Psychiatry* 50:263–269.
72. Harris LJ, Cárdenas RA, Spradlin MP Jr, Almerigi JB (2010): Why are infants held on the left? A test of the attention hypothesis with a doll, a book, and a bag. *Laterality* 15:548–571.
73. Beery AK, Lacey EA, Francis DD (2008): Oxytocin and vasopressin receptor distributions in a solitary and a social species of tuco-tuco (Ctenomys haigi and Ctenomys sociabilis). *J Comp Neurol* 507:1847–1859.
74. Campbell P, Ophir AG, Phelps SM (2009): Central vasopression and oxytocin receptor distributions in two species of singing mice. *J Comp Neurol* 516:321–333.
75. Baumgartner T, Heinrichs M, Vonlanthen A, Fischbacher U, Fehr E (2008): Oxytocin shapes the neural circuitry of trust and trust adaptation in humans. *Neuron* 58:639–650.
76. Stock S, Bremme K, Uvnas-Moberg K (1991): Plasma level of oxytocin during the menstrual cycle, pregnancy and following treatment with HMG. *Hum Reprod* 6:1056–1062.
77. Altemus M, Roca C, Galliven E, Romanos C, Deuster P (2001): Increased vasopressin and adrenocorticotropin responses to stress in the midluteal phase of the menstrual cycle. *J Clin Endocrinol Metab* 86:2525–2530.
78. Liedman R, Hansson LR, Howe D, Igidbashian S, McLeod A, Russell RJ, Akerlund M (2008): Reproductive hormones in plasma over the menstrual cycle in primary dysmenorrhea compared with healthy subjects. *Gynecol Endocrinol* 24:508–513.
79. Salonia A, Nappi RE, Pontillo M, Daverio R, Smeraldi A, Briganti A, *et al.* (2005): Menstrual cycle-related changes in plasma oxytocin are relevant to normal sexual function in healthy women. *Horm Behav* 47:164–169.
80. Naber F, Van IJzendoorn MH, Deschamps P, Van Engeland H, Bakermans-Kranenburg MJ (2010): Intranasal oxytocin increases fathers' observed responsiveness during play with their children: A double-blind within-subject experiment. *Psychoneuroendocrinology* 35:1583–1586.
81. Feldman R, Gordon I, Schneiderman I, Weissman O (2010): Natural variations in maternal and paternal care are associated with systematic changes in oxytocin following parent–infant contact. *Psychoneuroendocrinology* 35:1133–1141.