Toward a neuroscience of parenting : adult attachment and oxytocin affect neural and behavioral responses to infant attachment signals

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7 Oxytocin effects on complex brain networks are moderated by experiences of maternal love withdrawal

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Abstract

The neuropeptide oxytocin has been implicated in a variety of social processes. However, recent studies indicate that oxytocin does not enhance prosocial behavior in all people in all circumstances. Here, we investigate effects of intranasal oxytocin administration on intrinsic functional brain connectivity with resting state functional magnetic resonance imaging. Participants were 42 women who received a nasal spray containing either 16 IU of oxytocin or a placebo and reported how often their mother used love withdrawal as a disciplinary strategy involving withholding love and affection after a failure or misbehavior. We found that oxytocin changes functional connectivity between the posterior cingulate cortex (PCC) and the brainstem. In the oxytocin group there was a positive connectivity between these regions, whereas the placebo group showed negative connectivity. In addition, oxytocin induced functional connectivity changes between the PCC, the cerebellum and the postcentral gyrus, but only for those participants who experienced low levels of maternal love withdrawal. We speculate that oxytocin enhances prosocial behavior by influencing complex brain networks involved in self-referential processing and affectionate touch, most prominently in individuals with supportive family backgrounds.

Introduction

The neuropeptide oxytocin has been shown to stimulate a range of social behaviors (Bartz, Zaki, Bolger, & Ochsner, 2011; Carter, 1998; Insel, 2010). However, recent studies indicate that the beneficial effects of oxytocin are more nuanced than previously thought (Bakermans-Kranenburg, Van IJzendoorn, Riem, Tops, & Alink, 2012; Bartz et al., 2010; De Dreu et al., 2010; Van IJzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, & Tops, 2011). Contextual and individual differences seem to moderate oxytocin effects on social behavior and cognition (Bartz et al., 2011). In this study, we examine oxytocin effects on functional brain connectivity with resting state fMRI. In addition, we examine whether the effects of oxytocin on functional brain networks are moderated by experiences of maternal use of love withdrawal. Use of love withdrawal involves withholding love and affection when a child misbehaves or fails at a task. It has been associated with low self-esteem and low emotional well-being and has been found to moderate the positive effect of oxytocin on prosocial behavior (Van IJzendoorn et al., 2011). To our knowledge this is the first randomized controlled trial examining the neural mechanism underlying differential oxytocin effects with task-free techniques of functional brain networks in women.

Resting-state fMRI has become an important tool to study functional interactions in the human brain. Over the last decade many studies have found that spontaneous BOLD fluctuations are not random noise, but specifically organized in the resting human brain (Biswal et al., 2010). Regions that are functionally related tend to be highly correlated in their spontaneous BOLD activity during rest (Fox et al., 2007). Activity in the different resting state networks has been linked to different functions (Laird et al., 2011) and the degree of correlation has been shown to be related to behavioral outcomes (Vincent et al., 2006) and clinical conditions (Greicius, 2008). In addition, it has been shown that different drugs produce specific and detectable changes in these resting state networks (Khalili-Mahani et al., 2011; Tanabe et al., 2011). This indicates that resting state fMRI could be useful for "finger-printing" different pharmacological agents within the same individual's brain (Khalili-Mahani et al., 2011) as well as for studying differential pharmacological effects in individuals with different backgrounds.

The neuropeptide oxytocin plays a central role in attachment formation, affiliation and social behavior (Carter, 1998). Recent intranasal oxytocin administration experiments have shown that oxytocin stimulates sensitive parenting (Naber et al., 2010) and a range of other social behaviors (for a review see Bartz et al., 2011). However, oxytocin might not enhance prosocial behavior for all people in all circumstances. De Dreu et al. (2010) showed that oxytocin increases in-group altruism, but also increases defensive reactions to out-group members. Not all studies find these polarizing oxytocin effects; some indicate that the prosocial effects of oxytocin are hindered but not altered in individuals who experienced negative caregiving experiences (Bakermans-Kranenburg et al., 2012; Meinlschmidt & Heim, 2007). For example, Van IJzendoorn et al. (2011) found that oxytocin administration increased participants' willingness to donate money but only in participants who experienced low levels of parental love

withdrawal, without such effect in participants who experienced high levels of parental love withdrawal.

The experience of maternal love withdrawal thus appears to moderate the effects of oxytocin administration on prosocial behavior. Parental use of love-withdrawal has been associated with high concern over mistakes and low emotional well-being (Elliot & Thrash, 2004; Goldstein & Heaven, 2000; Renk, McKinney, Klein, & Oliveros, 2006). Excessive use of love withdrawal is considered psychological maltreatment (Euser, Van IJzendoorn, Prinzie, & Bakermans-Kranenburg, 2010) and has great impact on neurobiological development. Individuals who have experienced childhood maltreatment show atypical activation of the amygdala and frontal brain regions (Mehta et al., 2009; Van Harmelen et al., 2010). In addition, harsh caregiving experiences have been shown to affect the maturation of the cerebellum (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009), a brain region that is more dependent upon environmental factors than most other brain regions (Giedd, Schmitt, & Neale, 2007).

The underlying neural mechanism of oxytocin effects has been the focus of several neuro-imaging studies. Some studies showed that oxytocin increases activation in brain regions important for emotional processing, including the insula and inferior frontal gryus during the perception of social stimuli (Domes et al., 2010; Riem et al., 2011). Another target of oxytocin is the amygdala, a brain region implicated in the experience of fear, anxiety and arousal (MacLean, 1990). Oxytocin decreases amygdala activation during the perception of fear-inducing or aversive social stimuli; it has been suggested that this explains the stress-reducing effects of oxytocin (Gamer, Zurowski, & Buchel, 2010; Kirsch et al., 2005; Riem et al., 2011). The amygdala is part of a neural network involved in emotion processing and is strongly connected to other brain regions such as the precuneus/posterior cingulate cortex (PCC), the orbitofrontal cortex (OFC), the anterior cingulate (ACC) and the brainstem (Bos, Panksepp, Bluthé, & Honk, 2011; Pessoa, 2008). Consistent with other studies (Bos et al., 2012) a previous study showed that connectivity within this neural network can be enhanced by intranasal oxytocin (Riem et al., 2012), which in turn facilitates the integration of emotion and cognition and the evaluation of emotional signals (Pessoa, 2008).

In this study, we examined the influence of intranasally administered oxytocin on resting state functional connectivity in female twins. We used a seed based connectivity approach to reveal brain regions that are functionally connected with the amygdala, the insula and the PCC. These regions have a high degree of functional connectivity with other regions involved in emotional processing (Cauda et al., 2011; Cavanna, 2007; Cavanna & Trimble, 2006; Pessoa, 2008) and connectivities with these brain regions were affected by oxytocin in specific behavioral contexts (Gamer et al., 2010; Kirsch et al., 2005; Riem et al., 2011; Riem et al., 2012). Sripada et al. (2013) found intranasal oxytocin effects on resting state functional connectivity in males. It is as yet unknown whether oxytocin affects functional brain connectivity in a 'task-free' setting in females. In addition, we examined whether experiences of maternal love withdrawal moderates any oxytocin effects. We were especially interested in moderation of oxytocin effects on connectivity between the seed regions and the brainstem and the cerebellum,

because previous studies have shown that oxytocin modulates brainstem connectivity (Gamer et al., 2010; Kirsch et al., 2005) and that the maturation of the cerebellum can be affected by harsh caregiving experiences (Bauer et al., 2009).

Experimental procedures

Participants

Participants were selected from a larger study (Out, Pieper, Bakermans-Kranenburg, & Van IJzendoorn, 2010), see supporting information. A group of 44 right-handed females who met inclusion criteria and were willing to participate were recruited, 21 from MZ twin pairs and 23 from DZ twin pairs, without children of their own, in good health, without hearing impairments and MRI contraindications, pregnancy, and screened for psychiatric or neurological disorders and alcohol and drug use. The mean age of the participants was 28.98 years (*SD* =7.48, range 22-49). The majority of the participants (71.4 %) 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 (self-reported) menstrual cycle. Approximately 35 minutes before the start of the fMRI data acquisition subjects took 6 puffs of nasal spray containing oxytocin (16 IU total) or 6 puffs of a placebo-spray (NaCl solution) under supervision of the experimenter. Drug administration was double-blind. One sibling from each twin pair (9 MZ pairs, 7 DZ pairs) was randomly assigned to the oxytocin condition and the other sibling to the placebo condition, resulting in a group of 22 participants who were administered oxytocin and a group of 20 participants who were administered a placebo. See the supporting information for characteristics of the oxytocin and placebo group and for information about the scanning procedure. Participants were instructed to close their eyes during the entire resting state scan. After fMRI scanning participants completed a questionnaire on maternal use of love withdrawal.

Maternal love withdrawal

The questionnaire on maternal use of love withdrawal contained 11 items, all five items of the Withdrawal of Relations subscale of the Children's Report of Parental Behavior Inventory (CRPBI; (Beyers & Goossens, 2003; Schludermann & Schludermann, 1983)), two slightly adapted items from the same questionnaire, and four items adapted from the Parental Discipline Questionnaire (PDQ; (Hoffman & Saltzstein, 1967; Patrick & Gibbs, 2007). The 11-item questionnaire has been used previously (Van IJzendoorn et al., 2011) . Participants rated how well each of the 11 statements described their mother (e.g., "My mother is a person who, when I disappoint her, tells me how sad I make her") on a 5-point scale ranging from 1 (not at all) to 5 (very well). Cronbach's alpha was 0.85 in the current sample. The average item score on the love withdrawal questionnaire was 2.15 (*SD* = 0.71). The scores were normally distributed.

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. A T1-weighted anatomical scan was acquired (flip angle = 8°, 140 slices, voxelsize .875 × .875 × 1.2 mm). For resting state fMRI, a total of 170 T2*-weighted whole-brain EPIs were acquired (TR = 2.2 sec; TE = 30 msec, flip angle = 80°, 38 transverse slices, voxelsize 2.75 × 2.75 × 2.75 mm (+10% interslice gap)).

FMRI data analysis

Data analysis was carried out using FSL FEAT version 5.98 (Smith et al., 2004). Pre-statistics processing was applied before functional connectivity analyses, see supporting information. A seed based correlation approach was used for the current study (Fox & Raichle, 2007). As previous studies have shown that oxytocin affects insula and amygdala activation and connectivity (Domes et al., 2007; Gamer et al., 2010; Kirsch et al., 2005; Riem et al., 2011; Rilling et al., 2011), the amygdala and insula were selected as seed regions. In addition, the precuneus/posterior cingulate cortex (PCC), the main functional connectivity hub in the resting brain ((Tomasi & Volkow, 2011), was selected as seed region (center voxel = -2, -50, 36, see supporting information). Binary masks of the amygdala and insula (left and right) were created using the Harvard–Oxford (Sub)cortical Atlas. We extracted the mean time series for each participant from the left and the right amygdala, the left and the right insula and the PCC and applied separate models to analyze left and right amygdala, left and right insula and PCC connectivity. These times series were then used as a regressor in the model. In addition, the CSF signal and the global signal were added as regressors to the model in order to reduce the influence of artifacts caused by physiological signal sources on the results (Fox & Raichle, 2007). The temporal derivative of each regressor was added to the model resulting in 6 regressors in each model. Motion parameters were added to each model.

Contrasts of interest were the parameter estimates corresponding to the regressor of each of the seeds. These images represent the functional connectivity with the seed. First-level analyses were performed in native space. 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 using one-sample t-tests and group differences were tested using two-sample t-tests with the oxytocin versus placebo group comparison (Oxytocin > Placebo and Oxytocin < Placebo). We included age, menstrual cycle (follicular or luteal phase) and use of oral contraceptives as confound regressors in the model in all analyses. The statistical images were thresholded using clusters determined by $Z > 2.3$ and a cluster corrected significance threshold of $p < .05$. Mean Z values for significantly activated voxels within brain regions were calculated using FSL (FMRIb.ox.ac.uk/fsl/feat5/featquery.html) for visualization purposes.

Mean Z values for the brainstem and cerebellum (anatomically defined with the Harvard–Oxford Subcortical Atlas and the MNI Structural Atlas, average across

entire brainstem and cerebellum) were calculated for each participant (using FSL) in order to examine whether experiences of maternal love withdrawal moderate the effects of oxytocin. Hierarchical regression analyses were conducted to predict PCC-brainstem connectivity and PCC-cerebellum connectivity (residualized for age, menstrual cycle and use of oral contraceptives) with condition (oxytocin vs placebo) and experienced love withdrawal (centered) in the first step and the interaction between condition and love withdrawal in the second step. Two outlying values for PCC-cerebellum connectivity were winsorized to reduce any influence of extreme scores (Tabachnik & Fidell, 2001) .

Results

The analysis of PCC resting-state functional connectivity revealed a pattern of functional connectivity comprising the inferior and middle temporal gyrus, frontal pole, the superior frontal gyrus, the thalamus, brainstem and angular gyrus in the oxytocin and placebo group (see Figure 1 and Table s1 in supporting information). The between-group comparison (Oxytocin > Placebo) showed that oxytocin significantly induced connectivity changes between the PCC, the brainstem and the cerebellum (1 Cluster, size = 745 voxels, peak Z = 3.97, MNI coordinates x,y,z (mm) = -8, -6, -34) (see Figure 2). Inspection of the group means revealed that there was a positive connectivity between the PCC and the cerebellum and between the PCC and the brainstem in the oxytocin group and negative connectivities between these regions in the placebo group (see Figure 2), indicating that oxytocin changed PCC-brainstem and PCC-cerebellum connectivity. The analyses of resting-state functional connectivities with the amygdala and insula did not show significant group differences.

Hierarchical regression analyses were conducted to predict PCC-brainstem connectivity and PCC-cerebellum connectivity with condition (oxytocin vs placebo) and experienced love withdrawal in the first step and the interaction between condition and love withdrawal in the second step. The results of the hierarchical regression analyses are displayed in Table s2, see supporting information. For PCC-cerebellum connectivity the model was significant (F(3,38) = 3.89, p = 0.02). The effects of condition (ß = -0.27, p = 0.07) and love withdrawal (ß = -0.07, p = 0.61) were not significant. The interaction between condition and love withdrawal significantly predicted PCC-cerebellum connectivity (ß = 0.38, p = 0.01). To explore the interaction effect we created four groups: participants reporting high versus low love withdrawal in the oxytocin group and participants reporting high versus low love withdrawal in the placebo group (median split). A priori contrasts showed that oxytocin significantly changed PCC-cerebellum connectivity in participants with low love withdrawal scores (t(38) = 2.80, p = .01, Cohen's d = 1.49), but oxytocin did not have a significant effect in participants reporting high love withdrawal (t(38) = -0.25, p = .81, Cohen's d = -0.09), see Figure 3. For PCC-brainstem connectivity the overall model was also significant (F(3,38) = 3.15, p = 0.04). The effect of condition was significant (ß = -0.38, p = 0.01), but there was no effect of love withdrawal (ß = -0.07, p = 0.63) and no significant interaction effect (ß = 0.21, p = 0.17), see Table s2 in supporting information.

In a supplementary analysis, the whole brain analysis of PCC resting-state functional connectivity was repeated for participants reporting low love withdrawal. Again, the between-group comparison (Oxytocin > Placebo) showed that oxytocin significantly induced connectivity changes between the PCC, the brainstem and the cerebellum (Cluster size = 689 voxels, peak Z = 3.94, MNI coordinates x,y,z (mm) = 8, -34, -28). In addition, oxytocin significantly induced connectivity changes between the PCC and the postcentral gyrus (Cluster size = 581 voxels, peak Z = 4.11, MNI coordinates x,y,z (mm) = -8, -44, 60) (see Figure s1 in supporting information).

Discussion

In this study we explored the influence of oxytocin administration on intrinsic functional connections of complex brain networks and examined the moderating role of experienced maternal love withdrawal on effects of oxytocin in females. We found that oxytocin induced functional connectivity changes between the PCC and the brainstem. In addition, oxytocin induced functional connectivity changes between the PCC and the cerebellum and between the PCC and the postcentral gyrus, but only for participants who experienced low levels of maternal love withdrawal. Our results extend previous studies showing that positive oxytocin effects on behavior are lowered or absent in individuals who experienced negative caregiving experiences (Bakermans-Kranenburg et al., 2012; Meinlschmidt & Heim, 2007; Van IJzendoorn et al., 2011) and they indicate that quality of caregiving experiences moderates the effects of oxytocin even in the absence of social stimuli.

In our study, oxytocin effects were absent in individuals who experienced high levels of maternal love withdrawal but not altered, as in studies showing negative effects of oxytocin in some individuals under some circumstances (Bartz et al., 2010; De Dreu et al., 2010). In a previous study children who experienced early severe neglect did not show a change in oxytocin levels after physical contact with their mother, whereas oxytocin levels were increased in children who were reared in a loving family (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). The authors speculated that early adversity may alter the oxytonergic system fundamentally, possibly by influencing methylation in genetic areas regulating the oxytocin system (Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010). These differences in genetic expression may in turn lead to a decrease in sensitivity to intranasal oxytocin. This suggestion is supported by Meinlschmidts and Heim's (2007) study showing that subjects who experienced early parental separation exhibited attenuated cortisol decreases after intranasal oxytocin administration (versus placebo) compared with control subjects without early separation experiences, reflecting decreased sensitivity to the effects of oxytocin.

The PCC is considered a functional connectivity hub because of its high degree of connectivity with other brain regions (Buckner et al., 2009; Tomasi & Volkow, 2010). Our finding that intranasal oxytocin changes PCC connectivity is in line with a previous study in which we found that oxytocin increased connectivity

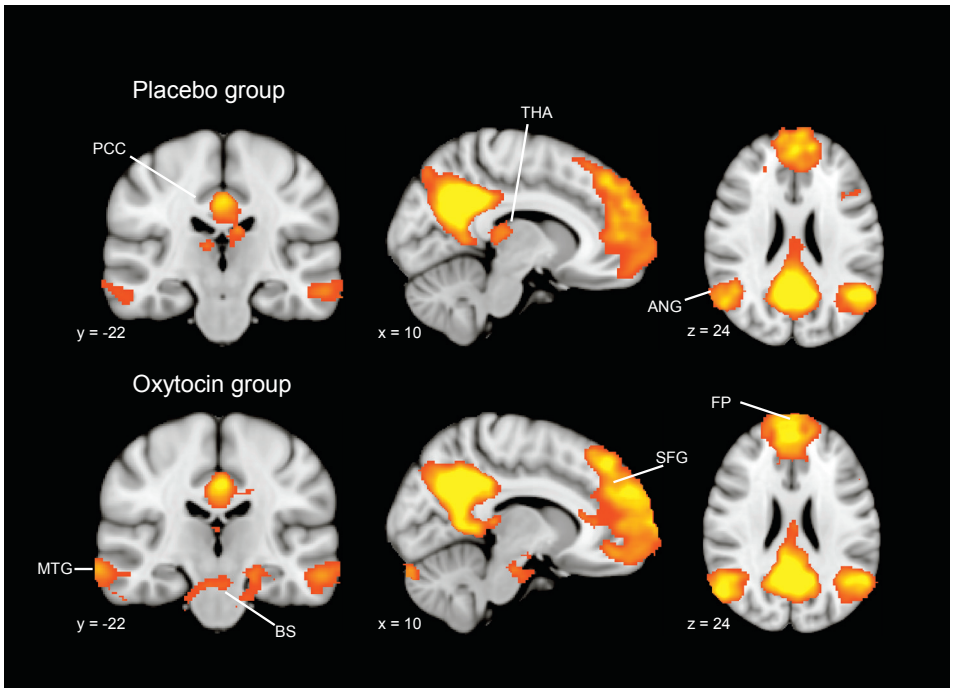

Figure 1. PCC resting-state functional connectivity in the placebo (top panel) and oxytocin group (lower panel), thalamus (THA), angular gyrus (ANG), middle temporal gyrus (MTG), superior frontal gyrus (SFG), frontal pole (FP), brainstem (BS). Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The right side of the brain corresponds to the left hemisphere and vice versa.

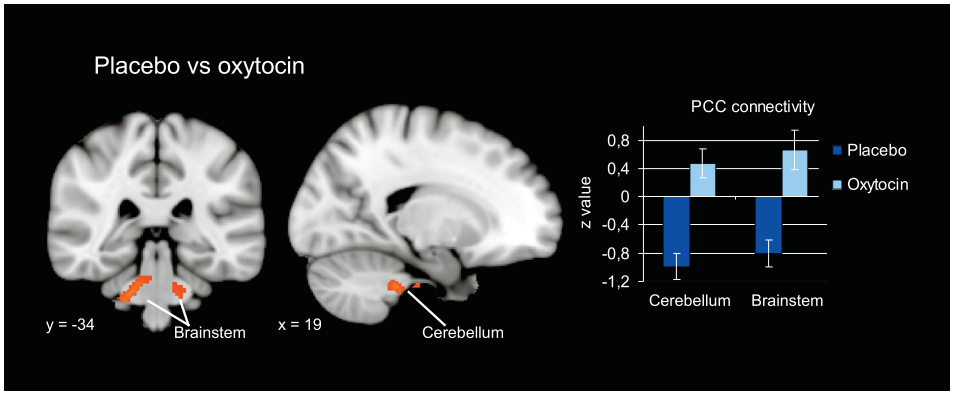

Figure 2. Group difference in PCC resting-state functional connectivity and mean *Z*-values for significantly activated voxels within the brainstem and cerebellum. Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The right side of the brain corresponds to the left hemisphere and vice versa.

between the amygdala and the PCC and other emotional brain regions during exposure to infant laughter (Riem et al., 2012). Enhanced PCC connectivity during rest may represent an increase in ongoing self-referential processes such as self-consciousness, sense of agency, and self-reflection (Cavanna, 2007; Cavanna & Trimble, 2006). According to simulation theory individuals use self-reflection to understand the mental states of others (Goldman, 1992). Therefore, the PCC and other regions in the default mode network have been suggested to be of great importance for social cognition (Schilbach, Eickhoff, Rotarska-Jagiela, Fink, & Vogeley, 2008). This suggestion is supported by studies pointing to a role of the PCC in understanding other people's minds (Wolf, Dziobek, & Heekeren, 2010). Our results are consistent with research showing that oxytocin is crucially involved in social cognition and affiliation and provide more insight into the neural mechanism underlying the beneficial effects of oxytocin.

The cerebellum has traditionally been associated with motor function and the coordination of movement. However, many studies indicate that it also plays an important role in emotion and cognition (Schmahmann, 2010; Stoodley, 2011). The cerebellum is connected with the dorsolateral prefrontal cortex, the PCC, the amygdala, the inferior parietal lobule and the brainstem (Heath & Harper, 1974; O'Reilly, Beckmann, Tomassini, Ramnani, & Johansen-Berg, 2010; Strick, Dum,

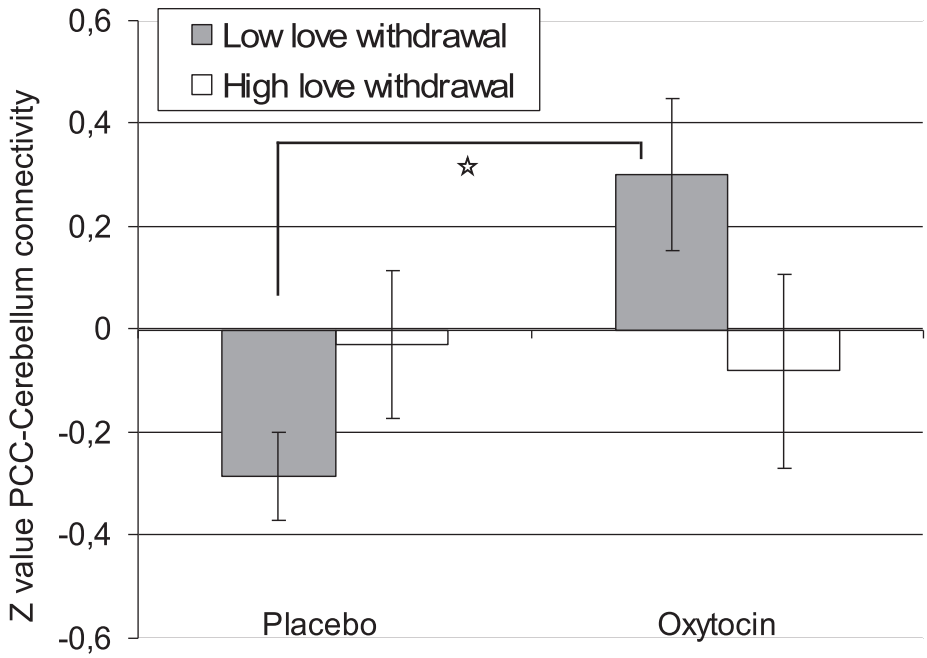

Figure 3 *Z*-values (M, SE) of PCC-cerebellum connectivity for participants reporting low versus high love withdrawal in the placebo group and participants reporting low and high love withdrawal in the oxytocin group. * $p < .05$

& Fiez, 2009) and studies have shown that these connectivities are important for cognition and emotion. For example, Alalade et al. (2011) showed that PCC-cerebellum connectivity (in a subregion of the cerebellum that was different from the significantly activated region of the cerebellum in the current study) is altered in patients with depression, and suggested that this could represent heightened rumination during resting state. The cerebellum is one of the least heritable brain structures and is more influenced by environmental factors during development than other brain regions (Giedd et al., 2007). This is in line with Bauer et al.'s (2009) study showing that children who experienced early deprivation had smaller superior-posterior cerebellar lobe volumes than a control group. The susceptibility of the cerebellum to environmental factors such as rejecting caregiving might partly explain the moderating role of maternal use of love withdrawal for oxytocin effects on connectivity between the PCC and the cerebellum.

In addition, we found that oxytocin induced connectivity changes between the PCC and the brainstem. Impaired PCC-brainstem coupling has been found in persistent vegetative state (Silva et al., 2010), indicating that connectivity between these regions plays a role in consciousness. Previous studies also found significant effects of oxytocin on brainstem connectivity. More specific, intranasal oxytocin administration decreased functional connectivity between the amygdala and the brainstem during exposure to fearful social faces (Gamer et al., 2010; Kirsch et al., 2005). Because projections between the amygdala and brainstem are involved in fear behavior and arousal (LeDoux, 2000) it has been suggested that decreased amygdala activation might be the underlying neural mechanism of the anxiolytic effects of oxytocin (Gamer et al., 2010; Huber, Veinante, & Stoop, 2005; Riem et al., 2011). However, in this study we did not find significant oxytocin effects on amygdala-brainstem connectivity, perhaps because we did not use fearful stimuli.

The oxytocin effects on increased functional connectivity between the PCC and the postcentral gyrus for individuals who experienced low levels of love withdrawal is consistent with studies showing that oxytocin levels are positively related with parent-infant contact and warm touch in married couples (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Holt-Lunstad, Birmingham, & Light, 2008). The postcentral gyrus is part of a somatosensory brain network (Tomasi & Volkow, 2011) that has been associated with the experience of pleasant and human touch (Hua et al., 2008; McCabe, Rolls, Bilderbeck, & McGlone, 2008). Our finding suggests that intranasal oxytocin leads to more efficient processing of touch-related information, but only in individuals with supportive family backgrounds. This is convergent with studies showing that oxytocin has an important role in initiating the "touch circuitry" between parents and infants in the first months of parenthood (Feldman et al., 2010; Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010) and with studies showing that this circuitry is disrupted in children who experienced early neglect (Fries et al., 2005).

Some limitations should be noted. We used a between-subject design which implies the risk of pre-existing differences between the oxytocin and placebo

group. However, most of our participants were monozygotic (MZ) and dizygotic (DZ) twin pairs, perfectly matched on age and global child-rearing experiences and even on genotype in MZ twin pairs. A limitation of our study is the use of self-reported maternal love withdrawal. Furthermore, conclusions regarding the direction of the relation between PCC, cerebellum and brainstem can not be made. In addition, we focused on functional connectivity between three regions of interest and the entire brain. Therefore our study does not allow conclusions on other region-to-region interactions. Lastly, our findings can only be generalized to women without parenting experience. Sripada et al. (2013) examined the effects of intranasal oxytocin on functional resting-state connectivity in males and found increased connectivity between the amygdala and rostral medial frontal cortex, but no effects on coupling between other brain regions. Oxytocin administration may thus have different effects on functional connectivity in men and women.

In conclusion, this is the first study to show intranasal oxytocin effects on complex brain networks in a task-free setting. We found that oxytocin changes functional connectivity between the PCC and the brainstem. In addition, oxytocin induced functional connectivity changes between the PCC, the cerebellum and the postcentral gyrus, but only for those participants who experienced low levels of maternal love withdrawal. Our study is the first to show that rejecting caregiving experiences moderate the effects of oxytocin in the absence of social stimuli. These findings support the suggestion that early social adversity can lead to a decrease in sensitivity to intranasal oxytocin by changing the oxytonergic system or its regulating genetic pathways maybe through methylation. Our results indicate that oxytocin enhances prosocial behavior by influencing complex brain networks involved in self-referential processing and affectionate touch, but they also show that part of these oxytocin induced connectivity changes are only brought about in individuals with supportive family backgrounds.

Supplementary material to: Oxytocin effects on complex brain networks are moderated by experiences of maternal love withdrawal

Participants
Participants were selected from a larger study investigating caregiving responses and physiological reactivity to infant crying (Out et al., 2010). The original sample consisted of 50 male and 134 female adult twin pairs.

Oxytocin versus Placebo
Twin siblings of 10 participants did not participate due to MRI contraindications or other exclusion criteria. Participants without a twin sibling were also randomly assigned to the oxytocin and placebo condition, resulting in a group of 22 participants (6 single MZ siblings) who were administered oxytocin and a group of 20 participants (3 single MZ siblings, 1 DZ sibling) who were administered a placebo. The sizes of the oxytocin and placebo group were different because of technical problems during data acquisition with 2 participants in the placebo group. Menstrual phase and use of oral contraceptives were balanced across the oxytocin and placebo condition: 12 participants in the oxytocin and 11 participants in the placebo group were in the luteal phase, whereas 8 participants in the oxytocin group and 8 participants in the placebo group were in the follicular phase. In all, 14 participants in the oxytocin group and 16 participants in the placebo group used oral contraceptives, whereas 8 participants in the oxytocin group and 4 participants in the placebo group did not use oral contraceptives. Mean displacement during resting state scanning did not differ for participants in the oxytocin and placebo group ($t(40) = -0.19$, $p = .85$). Love withdrawal scores did not differ for participants in the oxytocin or placebo condition ($t(40)=0.18$, $p = 0.86$). The correlation between love withdrawal reported by the twin siblings was $r = .51$ ($p < .05$).

Procedure
Time between oxytocin/placebo administration and data acquisition was similar to previous fMRI studies (e.g. Riem et al., 2011). Participants were instructed to comfortably position themselves on the scanner bed. Cushions were placed between the head coil and the participant in order to prevent head movement. The resting state scan was the first component of a longer fMRI scanning session, which decreased the likelihood of fatigue and sleepiness during resting state scanning. 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
The following pre-statistics processing was applied: motion correction (Jenkinson et al., 2002), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 6.0 mm, and high-pass temporal filtering

(highpass filter cutoff = 60.0 s). Functional scans were registered to the T1-weighted images, which were registered to standard space in order to calculate the transformation matrix for the higher-level group analysis (Jenkinson et al., 2002). The PCC seed region consisted of the peak location of posterior cingulate/precuneus hub (Buckner et al., 2009) and its adjacent voxels.

Table s1 MNI coordinates (mm), cluster size and peak *Z* values for significant clusters of functional connectivity.

		MNI coordinates				
Group	Region	x	y	z	Cluster size	Peak *Z*
Placebo	L Frontal Pole	-6	60	16	15326	7.39
	L Precuneus Cortex	-2	-50	36	12140	14.80
	L Angular Gyrus	-46	-60	28	3348	6.79
	L Middle Temporal Gyrus	-64	-44	-10	1648	4.52
	R Middle Temporal Gyrus	54	-16	-24	1404	4.76
Oxytocin	L Superior Frontal Gyrus	-4	46	32	17289	7.02
	L Precuneus Cortex	-2	-50	36	14409	14.90
	R Lateral Occipital Cortex	54	-68	24	3314	6.72
	L Postcentral Gyrus	-62	-12	-20	1682	5.09
	R Postcentral Gyrus	62	-14	-32	1663	5.71
	R Brainstem	2	-16	-14	1289	4.20
	R Cerebellum	26	-86	-30	608	5.36

$p < 0.05$, corrected by whole brain cluster threshold ($Z > 2.3$). Age, use of oral contraceptives and menstrual cycle were included as confound regressors in the model.

Table s2 Hierarchical regression analyses with condition (placebo/oxytocin), maternal love withdrawal and the interaction between condition and love withdrawal as predictors and mean *Z*-values of PCC-cerebellum connectivity and PCC-brainstem connectivity as outcomes.

	PCC-Cerebellum connectivity			PCC-Brainstem connectivity		
	B	ß	R^2	B	ß	R^2
Step 1			0.09			0.16
Condition (Oxytocin vs Placebo)	-0.27	-0.27		-0.43	-0.38*	
Love withdrawal	-0.05	-0.07		-0.06	-0.07	
Step 2			0.24			0.20
Condition x love withdrawal	0.55	0.38*		0.34	0.21	

* $p < .05$, Betas derived from the final block of the regression model.

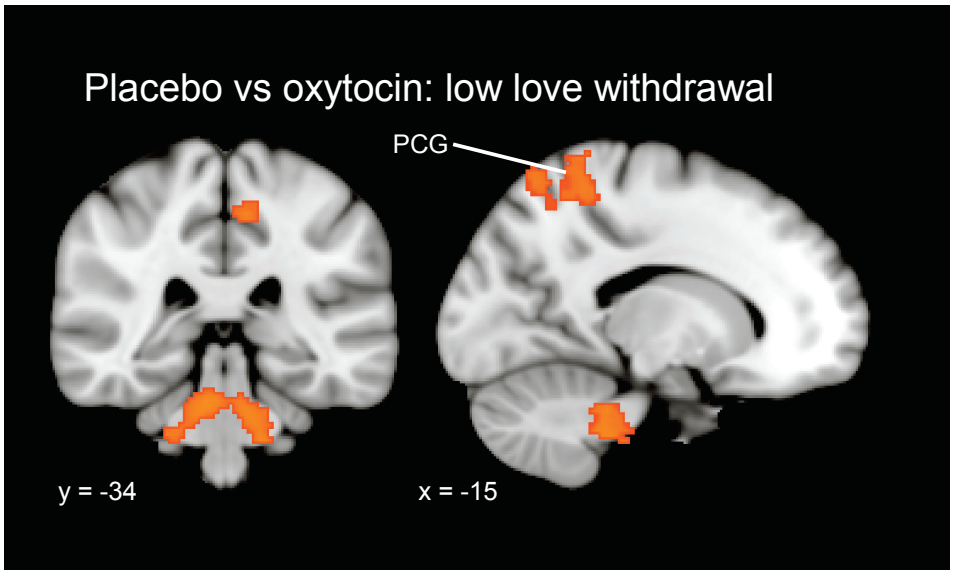

Figure s1. Group difference in PCC resting-state functional connectivity for participants reporting low love withdrawal, postcentral gyrus (PCG). Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The right side of the brain corresponds to the left hemisphere and vice versa.