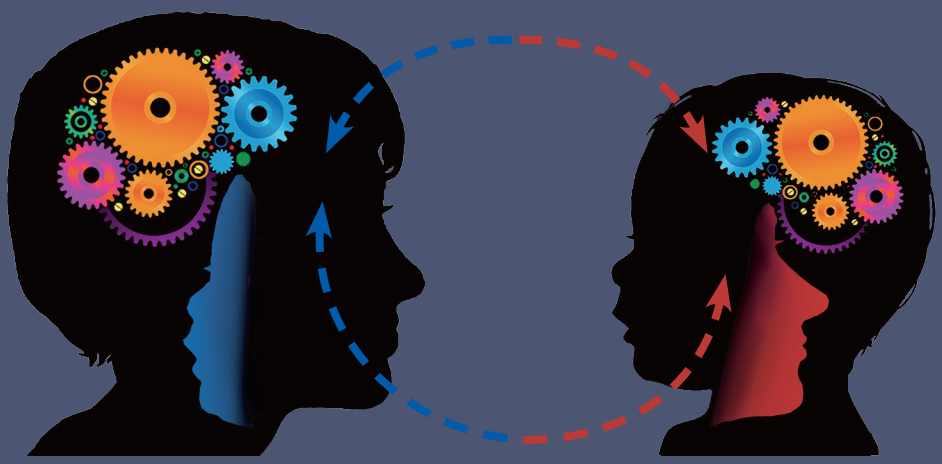
Toward a neuroscience of parenting : adult attachment and oxytocin affect neural and behavioral responses to infant attachment signals

Hendricx - Riem, M.M.E.

TOWARD A NEUROSCIENCE OF PARENTING

Adult attachment and oxytocin affect neural and behavioral responses to infant attachment signals



Madelon M.E. Hendricx-Riem

Toward a neuroscience of parenting

Adult attachment and oxytocin affect neural and behavioral responses to infant attachment signals.

Madelon M.E. Hendricx-Riem

Printed by Mostert & Van Onderen, Leiden

Cover design by Henk Riem and Madelon Hendricx-Riem, images: Shutterstock

Toward a neuroscience of parenting

Adult attachment and oxytocin affect neural and behavioral responses to infant attachment signals

Proefschrift

ter verkrijging van
de graad van Doctor aan de Universiteit Leiden,
op gezag van Rector Magnificus prof. mr. C.J.J.M. Stolker,
volgens besluit van het College voor Promoties
te verdedigen op dinsdag 4 juni 2013
klokke 15.00 uur
door

Madelon Mathilda Elisabeth Hendricx-Riem
geboren te Maastricht
in 1984

Promotiecommissie

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The studies described in the current thesis were supported by grants of the Netherlands Organization for Scientific Research (NWO) awarded to M.J. Bakermans-Kranenburg (VIDI grant no. 452-04-306; VICI grant no. 453-09-003), and M.H. van IJzendoorn (NWO SPINOZA prize).

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1 Introduction

As infants are fully dependent on their parents, correct perception and interpretation of infant signals is crucial for infant survival. It is therefore not surprising that specific brain circuits and neuroendocrine processes have evolved to perceive infant signals correctly and to respond adequately. However, parents vary in their ability to respond to their infants in a sensitive way and several factors may be involved in parental sensitive responsiveness. One important factor influencing parenting behavior is the neuropeptide oxytocin. Of all the hormones involved in parenting and other social behaviors, oxytocin has received the most interest, as evidenced by the high number of scientific studies over the past decade (Bos, Panksepp, Bluthé, & Honk, 2012; Van IJzendoorn & Bakermans-Kranenburg, 2012). Many studies suggest that oxytocin is related to sensitive parenting (Bakermans-Kranenburg & Van IJzendoorn, 2008; Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Weller, Zagoory-Sharon, & Levine, 2007), although the exact mechanism underlying this positive association is not entirely clear yet. Another factor that influences parenting behavior is adult state of mind with respect to attachment (Van IJzendoorn, 1995). In the current dissertation, the role of oxytocin and adult attachment in parenting is examined with a series of functional magnetic resonance imaging experiments.

Oxytocin: a neuroendocrine basis of social affiliation

The neuropeptide oxytocin is synthesized in the supraoptic and paraventricular nuclei of the hypothalamus and is released from the posterior pituitary into the bloodstream, for example in response to sexual stimulation, uterine dilatation, and nursing (Insel, 2010). It is crucially involved in the initiation of maternal care, attachment formation and several other forms of social behavior in rodents (Carter, 1998). The popularity of oxytocin, which is often labeled 'the love hormone', has increased exponentially the past decade, and so has the number of scientific studies investigating oxytocin's role in social cognition and behavior (Van IJzendoorn & Bakermans-Kranenburg, 2012). Correlational studies have shown that endogenous oxytocin is positively related to social behaviors, including human trustworthiness (Zak, Kurzban, & Matzner, 2005) and generosity in an ultimatum game (Barraza & Zak, 2009). Other studies found that intranasal administration of oxytocin stimulates a range of social behaviors (Graustella & MacLeod, 2012). It enhances trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), empathy (Bartz et al., 2010), emotion understanding (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007), cooperative behaviors (Rilling et al., 2012), and the familiarity of faces (Rimmele, Hediger, Heinrichs, & Klaver, 2009).

Recent research suggests that oxytocin also plays a role in parenting behaviors (Galbally, Lewis, Van IJzendoorn, & Permezel, 2011). Bakermans-Kranenburg and

Van IJzendoorn (2008) found that mothers with the presumably more efficient variant (GG) of the oxytonergic system gene (OXTR) showed higher levels of sensitive responsiveness, defined as the ability to accurately perceive children's signals and to respond in an adequate and prompt way (Ainsworth, Blehar, Waters, & Wall, 1978). In addition, Feldman, Weller, Zagoory-Sharon, and Levine (2007) showed that oxytocin levels across pregnancy and the postpartum period predict maternal behavior and the emotional bond with the infant. Moreover, oxytocin levels in fathers have been found to increase after stimulatory contact with their infant (Feldman et al., 2010), indicating that oxytocin is not only important for maternal behavior but also for fathering. However, these studies were correlational and do not provide evidence for a causal role of oxytocin in human parenting. The study by Naber, Van IJzendoorn, Deschamps, Van Engeland, and Bakermans-Kranenburg (2010) was one of the first experiments to investigate the role of oxytocin in parenting. Naber et al. found that intranasal oxytocin administration leads to more responsive interactions of fathers with their child during play, thus providing experimental evidence for a causal role of oxytocin in elevating the level of paternal responsiveness.

Although many studies show that oxytocin plays an important role in parenting, it is not yet clear *how* oxytocin influences parenting behaviors. One way in which oxytocin might influence parenting behaviors is by affecting the perception of infant signals. Infant crying is one of the most salient attachment signals. It enhances infant survival by eliciting parental proximity and care and by conveying information on the health condition of the child (Bowlby, 1969/82; Soltis, 2004). It has been described as a graded signal that changes as a function of the level of distress of the infant (Gustafson, Wood, & Green, 2000). For example, infants who are in pain cry at higher fundamental frequencies than infants who are hungry (Soltis, 2004). Parents are sensitive to the acoustics of crying and use this information in combination with contextual clues to select an adequate caregiving response. On the other hand, crying also elicits negative emotions such as aversion and anger (Dix, 1991; Dix, Gershoff, Meunier, & Miller, 2004) and excessive infant crying can even trigger child abuse and neglect (Soltis, 2004). In the Netherlands, six months after the infant's birth nearly 6% of the parents report that they have shaken, smothered, or slapped their infant in order to stop the crying (Reijneveld, Van der Wal, Brugman, Sing, & Verloove-Vanhorick, 2004). Because excessive infant crying has been found to be one of the major triggers of child abuse and neglect, examining the mechanisms that are involved in reactions of adults to infant crying is crucial. Individual differences in oxytocin levels may explain why some parents remain sensitive whereas other parents lack the empathic ability to abstain from harsh or even abusive responses their infant's crying.

Oxytocin might also influence parenting behaviors by affecting the perception and processing of happy infant signals such as infant laughter. Laughter is suggested to be the outcome of a long evolutionary history (Van Hooff, 1972), and its production as well as perception might be hardwired in human beings (Owren & Bachorowski, 2003). Infant smiling and laughing are basic attachment behaviors that create closer proximity to a caregiver, thereby enhancing infant

survival (Bowlby, 1969/82; Sroufe & Waters, 1976). Infant laughter is a uniquely rewarding experience for parents and it activates neural reward centers in the parental brain (Kringelbach et al., 2008; Strathearn, Li, Fonagy, & Montague, 2008). However, not all parents may perceive their laughing or smiling infant as a reward. Mothers with a postpartum depression are less responsive to their infants, show less positive affect and are less accurate in identifying happy infant stimuli (Arteche et al., 2011; Reck et al., 2004), possibly because of the malfunctioning of reward centers in the brain (Moses-Kolko et al., 2011). Low oxytocin levels may be involved in the reduced rewarding value of infant stimuli in depressed mothers, as oxytocin may link social cues, such as infant laughter, with dopaminergic brain regions involved in reward processing (Strathearn, Fonagy, Amico, & Montague, 2009).

Some neuro-imaging studies suggest that oxytocin might sensitize caregivers to infant crying and laughter by modulating neural circuits related to the perception of infant signals. For example, mothers who experienced childbirth by vaginal delivery showed a unique pattern of neural responses to their own infant's crying sounds as compared to mothers who had a cesarean section delivery (Swain et al., 2008). The difference in brain responses to crying between mothers who gave birth through vaginal delivery and cesarean section may be explained by the increase in oxytocin release that occurs after vaginal delivery but not after cesarean section. Furthermore, Bos, Hermans, Montoya, Ramsey, and Van Honk (2010) found that testosterone administration increased activation in the thalamocingulate region, insula, and the cerebellum in response to crying, possibly by influencing the oxytocin system. However, the exact influence of oxytocin on neural responding to infant signals is still unknown. In Chapter 2 and 3, we try to shed more light on this topic by investigating the effects of intranasal oxytocin administration on neural responses to infant crying and laughter, measured with functional Magnetic Resonance Imaging (fMRI). These chapters aim to provide more insight into the mechanism underlying the positive effects of oxytocin on parenting.

Adult attachment: predictor of responses to infant crying

Adult attachment has been shown to be another important factor influencing sensitive responsiveness to infant crying. Adult attachment reflects the current state of mind with respect to attachment and refers to the mental representation of past and present attachment experiences. The Adult Attachment Interview (AAI) is considered the "gold standard" for the measurement of adult attachment (George, Kaplan, & Main, 1985; Hesse, 2008; Main & Goldwyn, 1984). It is an hour-long interview in which participants are asked about their childhood attachment experiences with their parents and how they think they were affected by these experiences (Hesse, 2008; Main & Goldwyn, 1984). Coding of the AAI yield three major attachment classifications: Secure-Autonomous, Insecure-Dismissing, and Insecure-Preoccupied. Individuals who are classified as secure-autonomous value attachment relationships, describe their attachment experiences (whether positive or negative) coherently, and consider them influential in their personality development. Adults classified as insecure-dismissing, however,

are uncomfortable with the topic of the interview. They idealize their childhood experiences or tend to minimize the importance of attachment in their own lives. Adults with a preoccupied classification show active anger towards attachment figures and tend to emphasize the influence of their attachment experiences.

Research has shown that adult attachment influences parental responding to infant crying. Secure parents are suggested to be able to respond adequately to their crying infants since they are free of distorted perceptions of their infants' needs (Ainsworth et al., 1978). Insecure parents, on the other hand, are less accurate at identifying infant emotions and tend to make negative, internal attributions to the nature of the crying (e.g., the child is spoiled or has a difficult temperament) (Leerkes & Siepak, 2006). In addition, an fMRI study showed that insecure mothers show increased activation in a brain region associated with feelings of unfairness and disgust while looking at pictures of their own infant's sad face, whereas secure mothers showed activation of reward areas (Strathearn et al., 2009). Thus, insecure parents seem to perceive attachment-related information in a defensive and negatively biased manner. This kind of information processing contributes to insensitive parental caregiving, which can in turn result in insecure infant attachment (Dykas & Cassidy, 2011). It is therefore of great importance to understand the mechanism underlying the negative perception of infant signals in individuals with insecure attachment representations. Chapter 4 sheds more light on this mechanism by focusing on neural, emotional and behavioral responding to infant crying in adults with different attachment representations.

Oxytocin: context and person matter

Many studies on the beneficial effects of oxytocin on social cognition and behavior have been published over the past ten years (for review see Graustella & Macleod, 2012; Van IJzendoorn & Bakermans-Kranenburg, 2012). However, recent research indicates that the effects of oxytocin are more nuanced than previously thought (Bartz, Zaki, Bolger, & Ochsner, 2011). Contextual factors and individual differences seem to moderate the effects of oxytocin on social cognition. For example, the trust-enhancing effects of oxytocin disappear when partners are unknown or believed to be unreliable (Declerck, Boone, & Kiyonari, 2010; Mikolajczak et al., 2010). In a series of experimental studies De Dreu et al. (2010) showed that oxytocin can even have negative effects on social behavior. Intranasal oxytocin enhanced in-group altruism, but at the same time increased defensive reactions toward out-group members, indicating that oxytocin may drive a 'tend and defend' response. Social context may thus be crucial in shaping the effects of oxytocin on social cognition. It is currently unknown whether and how context may modulate the effects of oxytocin on parental behaviors. Chapter 5 addresses this issue by focusing on the effects of contextual factors on the influence of oxytocin on neural responding to infant crying.

In addition to contextual factors, individual differences may also be involved in shaping the effects of oxytocin on social behaviors. For example, Bakermans-Kranenburg, Van IJzendoorn, Riem, Tops, and Alink (2012) found that intranasal oxytocin decreased the use of excessive handgrip force in response to infant crying, but only in individuals who did not experience harsh discipline. Similarly, Van

IJzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, and Tops (2011) found that intranasal oxytocin administration enhanced donating to a charity, but only in individuals who had experienced low levels of love withdrawal, a parental strategy that involves withholding love and affection when a child misbehaves or fails at a task. These findings indicate that unfavorable caregiving experiences moderate the beneficial effects of oxytocin. In Chapter 6 we investigate the effects of intranasal oxytocin on prosocial helping behavior toward an excluded person during a virtual ball-tossing game called Cyberball (Williams & Jarvis, 2006), taking into account experiences of maternal love withdrawal as a potential moderator. Cyberball has been used in many studies to examine the effects of being excluded (Crowley, Wu, Molfese, & Mayes, 2010; Gonsalkorale & Williams, 2007; Harmon-Jones, Peterson, & Harris, 2009; Zadro, Williams, & Richardson, 2004). So far, few studies investigated the way individuals respond when they observe someone else being excluded.

An important research tool that can be used to study the influence of individual differences on the effects of oxytocin is resting state fMRI. The brain consumes about 20% of the body's energy during rest, partly because of spontaneous neuronal activity (Fox & Raichle, 2007). This resting-state energy consumption is very high when compared with task-related increases in neuronal metabolism (<5%), suggesting that intrinsic activity is far more important than evoked activity in terms of overall brain function (Raichle, 2009). Although spontaneous neuronal activity consumes a high amount of the brain's energy, these spontaneous fluctuations have been viewed as noise for many years. This view changed when studies found that spontaneous blood oxygenation level dependent (BOLD) fluctuations are not random noise, but specifically organized in the resting human brain (Biswal et al., 2010). Smith et al. (2009) found that regions that are functionally related tend to be highly correlated in their spontaneous BOLD activity during rest. The degree of correlation has been shown to predict behavioral outcomes (Vincent et al., 2006) and is related to clinical conditions (Greicius, 2008), such as dementia (Hafkemeijer, van der Grond, & Rombouts, 2011), depression (Veer et al., 2010), schizophrenia (Zhou et al., 2007) and autism (Cherkassky, Kana, Keller, & Just, 2006).

Interestingly, it has been shown that drugs produce specific and detectable changes in these resting state networks (Khalili-Mahani et al., 2012; 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., 2012) as well as for studying differential pharmacological effects (e.g., effects of oxytocin administration) in individuals with different backgrounds. Little research has been conducted on the effects of pharmacological treatments on brain function and connectivity. Moreover, it is unknown how unfavorable caregiving experiences influence the effects of intranasal oxytocin on resting state at the neural level. This is investigated in Chapter 7, which focuses on the effects of intranasal oxytocin administration on functional brain connectivity during rest, taking into account experiences of maternal love withdrawal as a potential moderator. Examining the effects of oxytocin on brain connectivity during rest is especially interesting because it provides more insight into the influence of

oxytocin on the baseline state of the brain, in the absence of stimuli or any social context.

Focus and outline of the present thesis

The general aim of the current thesis is to gain more insight into the associations between oxytocin, adult attachment and parenting behaviors, and into the role of context and family background in shaping oxytocin effects. The thesis presents a series of functional magnetic resonance imaging studies that shed more light on the effects of intranasal oxytocin administration on brain activation and connectivity. The studies presented in Chapter 2, 3, 4, and 7 are based on a sample of female twin pairs (aged 22-49 years) without children of their own. The studies presented in Chapter 5 and 6 are based on a different sample of women (aged 18-27 years), again without children of their own. Women without children of their own were selected for participation in these studies in order to reduce hormonal influences associated with differences in parental experience and between men and women, because of the lack of studies investigating oxytocin effects in women (Bos et al., 2012), and because it is especially maternal behavior that affects child developmental outcomes (Cabrera, Fagan, Wight, & Schadler, 2011).

Figure 1 presents a graphic representation of the topics of the current dissertation. In the first part of the thesis we aim to clarify the mechanism underlying the positive association between oxytocin and parenting. More specifically, we examine the effects of intranasal oxytocin administration on

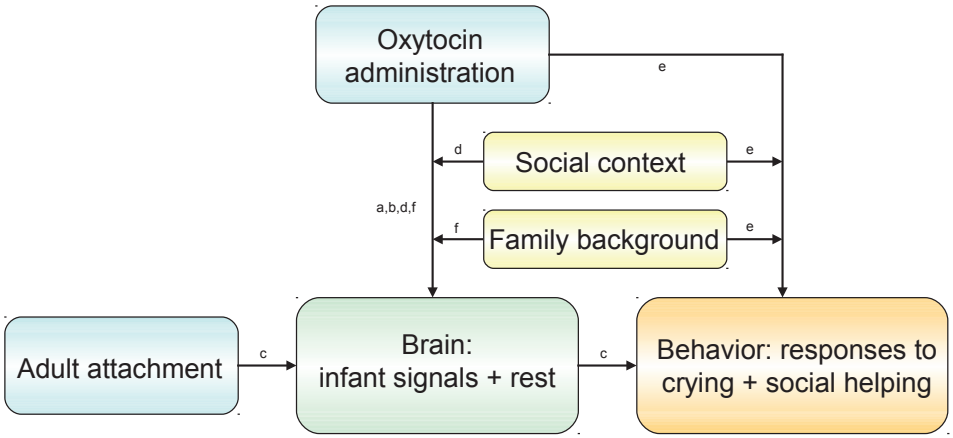

Figure 1. Graphic representation of the topics of the current dissertation. In Chapter 2 and 3 we examine the influence of oxytocin on brain responses to infant crying (a) and laughter (b). In Chapter 4, a study about the influence of adult attachment representation on neural, emotional and behavioral responding to infant crying is presented (c). Chapter 5 focuses on the influence of social context on the effects of oxytocin on brain responses to infant crying (d). In Chapter 6 and 7 we examine oxytocin effects on social helping behavior to an excluded known person with a sad or neutral facial expression (e) and on brain connectivity during rest (f), taking into account family background as a potential moderating factor.

neural responses to infant crying (Chapter 2, a in Figure 1) and laughter (Chapter 3, b in Figure 1) with a randomized controlled trial with female twin pairs. Chapter 4 addresses the influence of adult attachment, measured with the Adult Attachment Interview, on neural, emotional and behavioral responses to infant crying (c in Figure 1). In Chapter 5, 6 and 7 we aim to gain more insight into the influence of contextual factors and individual differences on the effects of oxytocin on parenting and social behavior. Chapter 5 focuses on the influence of intranasally administered oxytocin on the perception of infant crying in systematically varied contexts (d in Figure 1). We examine differential effects of oxytocin on neural responding to crying that was indicated as coming from a sick infant and crying coming from a bored infant. Chapter 6 presents a randomized-controlled trial which investigates the effects of oxytocin administration on prosocial behavior during Cyberball (e in Figure 1). Effects of intranasal oxytocin administration on prosocial helping behavior toward an excluded known person with a neutral or a sad facial expression were examined, taking into account experiences of love withdrawal as a potential moderator. Chapter 7 focuses on the influence of harsh caregiving experiences on the effects of intranasal oxytocin at the neural level (f in Figure 1). Resting state fMRI was used to explore the effects of oxytocin on functional brain connectivity in a 'task-free' setting, again taking into account experiences of love withdrawal as a potential moderator. In the concluding chapter, the findings of the studies are discussed and implications for future research are presented. Lastly, the appendix presents a study in which we investigated the role of oxytocin in parenting from a different point of view, namely by examining the influence of OXTR genotype as well as depressive symptoms on heart rate responses to infant crying.

2 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, & Serge A.R.B. Rombouts (2011). Biological Psychiatry 70, 291-297.

Abstract

Oxytocin facilitates parental caregiving and mother-infant bonding and might be involved in responses to infant crying. Infant crying provides information about the infant's physical status and mood, 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. In a randomized controlled trial we investigated the influence of intranasally administered oxytocin on neural responses to infant crying using functional Magnetic Resonance Imaging (fMRI). Blood oxygenation level dependent (BOLD) responses to infant crying were measured in 21 women who were administered oxytocin and 21 women who were administered a placebo. Experimentally induced oxytocin levels reduced activation in the amygdala and increased activation in the insula and inferior frontal gyrus pars triangularis. 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.

Introduction

Infant crying alerts parents to the needs of the infant and elicits parental proximity and caregiving (Bowlby, 1969; Zeifman, 2001). Since 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 (Bos, Hermans, Montoya, Ramsey, & Van Honk, 2010; Seifritz et al., 2003). Oxytocin is a neuropeptide that facilitates parental caregiving and mother-infant bonding in various species, including humans (Carter, 1998; Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Insel, 2010). 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 caregivers' responsiveness. In this study, we examine the effects of experimentally elevated levels of oxytocin on neural responses to infant crying. As crying has been found to be one of the major triggers of child abuse and neglect (Reijneveld, Van der Wal, Brugman, Sing, & Verloove-Vanhorick, 2004), examining the mechanisms that are involved in adults' reactions to infant crying is crucial.

Animal studies have shown that oxytocin is involved in lactation, pregnancy and the onset of maternal behavior (Carter, 1998; Insel, 2010). 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 (Feldman et al., 2007). In addition, intranasally administered oxytocin has been shown to stimulate a range of social behaviors, including empathy (Bartz et al., 2010; Hurlemann et al., 2010), mind-reading (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007), trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), and in-group altruism (De Dreu et al., 2010). Carter (1998) argued that oxytocin may 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 Heinrichs and Domes' suggestion that oxytocin plays an important role as an underlying neurobiological mechanism for the anxiolytic/stress-protective effects of positive social interaction (Heinrichs & Domes, 2008). On the genetic level individuals with the potentially more efficient variant (GG) of the oxytonergic receptor gene (OXTR rs53576) show reduced levels of stress and increased levels of empathy (Rodrigues, Saslow, Garcia, John, & Keltner, 2009), as well as more sensitive parental interactions with toddlers (Bakermans-Kranenburg & Van IJzendoorn, 2008).

An important component of parental sensitive caregiving is responsiveness to infant crying behavior. Infant crying provides information about the infant's physical health and mental state, and the intensity of distress can be derived from the acoustics of the cry sound (Gustafson, Wood, & Green, 2000; Murray, 1979). The association between oxytocin and increased parental sensitivity may be partly due to the influence of oxytocin on responses to infant crying (Riem, Pieper, Out, Bakermans-Kranenburg, & Van IJzendoorn, 2011). 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 (Lorberbaum et al., 2002; Seifritz et al., 2003; Swain et al., 2008). The thalamus is considered important for mammalian mother-infant attachment behavior (MacLean, 1990). The insula is involved in maternal bonding and an important region implicated in empathy and emotion understanding (Bartels & Zeki, 2004), and it has been suggested that oxytocin is involved in the neurochemical mechanism underlying emotional empathy (Hurlemann et al., 2010; Shamay-Tsoory, 2011). Bos et al. (2010) 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 oxytonergic system. Furthermore, vaginal delivery leads to increased oxytocin levels following vaginal-cervical stimulation, and mothers who experienced childbirth by vaginal delivery showed more brain activation in the superior temporal gyrus, the insula and the anterior cingulate gyrus compared to mothers who had a cesarean section delivery (Swain et al., 2008).

While oxytocin seems to increase activation in brain regions involved in empathy (Shamay-Tsoory, 2011), it decreases activation in the neural circuitry for anxiety and disgust (Gamer, Zurowski, & Buchel, 2010; Stark et al., 2003). Kirsch et al. (2005) 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 (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; McCarthy, McDonald, Brooks, & Goldman, 1996). Mothers experience strong emotional reactions to infant crying, ranging from empathy to anxiety and anger (Dix, 1991; Out, Pieper, Bakermans-Kranenburg, Zeskind, & Van IJzendoorn, 2010). Aversion or other negative emotional reactions may lead to insensitive parenting behaviors such as withdrawal or hostility in order to stop (having to listen to) infant crying (Dix, Gershoff, Meunier, & Miller, 2004); and high-pitched infant crying in particular is an important trigger for child abuse (Out, Pieper, Bakermans-Kranenburg, Zeskind, et al., 2010; Soltis, 2004). As oxytocin decreases activation in the neural circuitry for anxiety and aversion, it might prevent parents from becoming overreactive to the disturbing infant cry sounds. Although several studies have shown that oxytocin decreases amygdala reactivity to fearful stimuli in males, only one study has been conducted on the effects of oxytocin administration in females. Domes et al. (2010) found that in females intranasal oxytocin increased amygdala activation in response to angry and happy adult faces, which is in contrast with reported effects in males (Kirsch et al., 2005). However, oxytocin may reduce amygdala activation in females listening to infant crying which would be in line with the stress-reducing effects of endogenous oxytocin release in breast-feeding females (Heinrichs et al., 2001; Heinrichs, Neumann, & Ehlert, 2002).

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 since 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 (Porter, Porges, & Marshall, 1988; Soltis, 2004; Zeskind & Collins, 1987). Blood oxygenation level dependent (BOLD) response to infant crying was measured using functional Magnetic Resonance Imaging (fMRI). Whole brain analysis was performed to explore the neural effects of oxytocin. In particular, we expected that oxytocin administration would be related to increased activity in the thalamocingulate circuit and in the insula, and decreased activity in the amygdala. Regions of interest (ROI) analyses were conducted to examine the effects of oxytocin in these regions.

Method

Participants

Participants were selected from a larger study investigating caregiving responses and physiological reactivity to infant crying (Out, Pieper, Bakermans-Kranenburg, & Van IJzendoorn, 2010). The original sample consisted of 50 male and 134 female adult twin pairs. Zygosity was determined on the basis of a zygosity questionnaire (Magnus, Berg, & Nance, 1983) and additional genetic analysis of selected polymorphisms. A group of 43 right-handed females 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 and MRI contraindications, pregnancy, psychiatric or neurological disorders, and screened for alcohol and drug use. One dizygotic 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 thirty-six minutes 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 (Marsh, Yu, Pine, & Blair, 2010; Rimmele, Hediger, Heinrichs, & Klaver, 2009). 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. Participants were instructed to attend to the sounds they would hear. Before drug administration and after fMRI scanning participants completed a mood questionnaire in order to track mood changes following drug administration. Participants rated on 7-point Likert scales how much anxiety, anger, frustration, empathy, happiness and calmness they felt (Kirsch et al., 2005). In addition, they rated the perceived urgency of

the infant cry sounds on a 5-point Likert scale after fMRI scanning (Zeskind & Marshall, 1988).

Cry paradigm

Cry sounds were derived from the spontaneous crying of a healthy 2-day old infant. A 10-s portion of the sustained period of crying was selected. The peak fundamental frequencies (Peak F0) of the entire cry were 515 ± 15Hz. Two new 10-s 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 (Dessureau, Kurowski, & Thompson, 1998; Out, Pieper, Bakermans-Kranenburg, Zeskind, et al., 2010; Schuetze & Zeskind, 2001; Schuetze, Zeskind, & Das Eiden, 2003). 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 8 cycles, each cycle consisting of 6 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 s (see Fig 1).

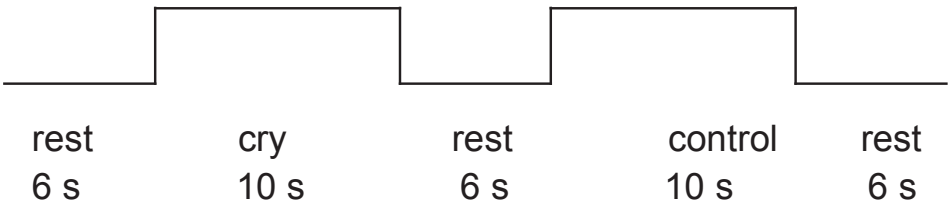

Figure 1. The cry paradigm. Cry sounds and control sounds were presented for 10 seconds, followed by a rest period of 6 seconds.

Oxytocin versus placebo

One sibling from each twin pair was randomly assigned to the oxytocin condition and the other sibling to the placebo condition. Participants without a twin sibling were also randomly assigned to the oxytocin and placebo condition. Approximately thirty-six minutes before the start of the fMRI data acquisition subjects took 6 puffs of nasal spray containing oxytocin (16 IU total, RVG number 03716, Sandoz b.v.) or 6 puffs of a placebo-spray (NaCl 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, voxelsize .875 × .875 × 1.2 mm). For fMRI, a total of 360 T2*-weighted whole-brain EPIs were acquired (TR = 2.2sec; TE = 30 msec, flip angle = 80°, 38 transverse slices, voxelsize 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 using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.FMRIb.ox.ac.uk/fsl; (Smith et al., 2004)). The following pre-statistics processing was applied: motion correction (Jenkinson & Smith, 2001), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 5.0 mm, and high-pass temporal filtering (highpass filter cutoff = 50.0s). Functional scans were registered to T1-weighted images, which were registered to standard space (Jenkinson, Bannister, Brady, & Smith, 2002; Jenkinson & Smith, 2001).

In native space, functional activation was examined using 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 gamma hemodynamic response function and its temporal derivative was added to the model, giving 12 predictors. In order to identify regions involved in the perception of infant crying the following contrasts were assessed: 1) $\text{Cry}_{\text{combined 500, 700, 900 Hz}} > \text{Control}_{\text{combined 500, 700, 900 Hz}}$, 2) $\text{Cry}_{\text{500 Hz}} > \text{Control}_{\text{500 Hz}}$, 3) $\text{Cry}_{\text{700 Hz}} > \text{Control}_{\text{700 Hz}}$, 4) $\text{Cry}_{\text{900 Hz}} > \text{Control}_{\text{900 Hz}}$. 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 we tested for group differences using two-sample t-tests on these contrasts with the oxytocin versus placebo group comparison ($\text{Oxytocin}^{\text{Cry > Control}} > \text{Placebo}^{\text{Cry > Control}}$). Similar to Domes et al. (35) we also examined reduced brain activation in the oxytocin group compared to the placebo group in the reverse contrast ($\text{Oxytocin}^{\text{Cry > Control}} < \text{Placebo}^{\text{Cry > Control}}$). The statistical images were thresholded using clusters determined by $Z > 2.3$ and a cluster corrected significance threshold of $p < .05$ (Worsley, 2001).

Additionally, regions of interest (ROI) analyses were performed with FSL 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 (Bos et al., 2010; LeDoux, 2000; Seifritz et al., 2003) and were defined using the Harvard–Oxford cortical atlas (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). ROI analyses were limited to these search regions, applying the same statistical threshold as for the whole brain analyses, however 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 using Featquery (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 monozygotic twin/dizygotic 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 using Featquery for visualization purposes.

Results

Statistical images were thresholded using 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 (see Table 1 and Fig. 2). 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 to the control sounds. The thalamus and insula did not show a significant difference in activation during infant crying compared to 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 (see Table 1). 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 compared to 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 compared to the 900 Hz control sound in the whole brain analysis (contrast 4). The regions of interest 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 $^{\text{Cry > Control}}$ > Placebo $^{\text{Cry > Control}}$ and Oxytocin $^{\text{Cry > Control}}$ < Placebo $^{\text{Cry > Control}}$). These contrasts did not yield significantly different activation in the whole brain analysis. However, ROI analysis showed that compared to the placebo group, participants who received oxytocin showed reduced activation in the

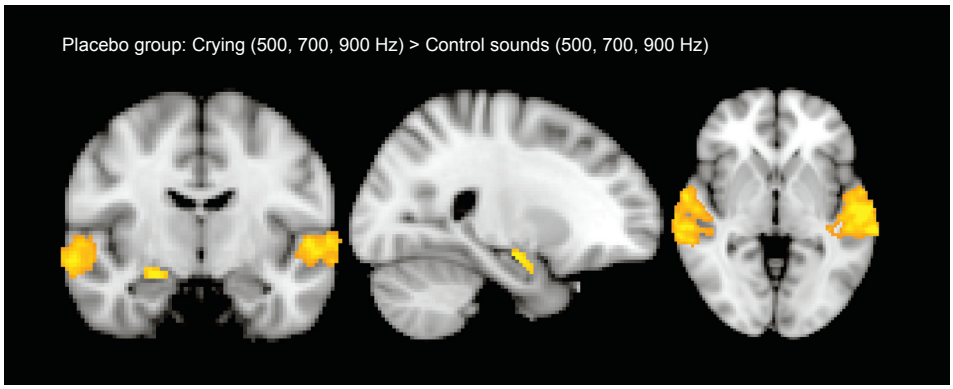

Figure 2. Significant activation in the bilateral superior and middle temporal gyrus and the amygdala for the contrast Crying (500, 700, 900 Hz) > Control sounds (500, 700, 900 Hz). Statistical images were thresholded using clusters determined by $Z > 2.3$ and a cluster corrected significance threshold of $p < 0.05$. The amygdala activation was revealed by ROI analysis limited to this search region, applying the same statistical threshold, however correcting only for the size of the ROI volume.

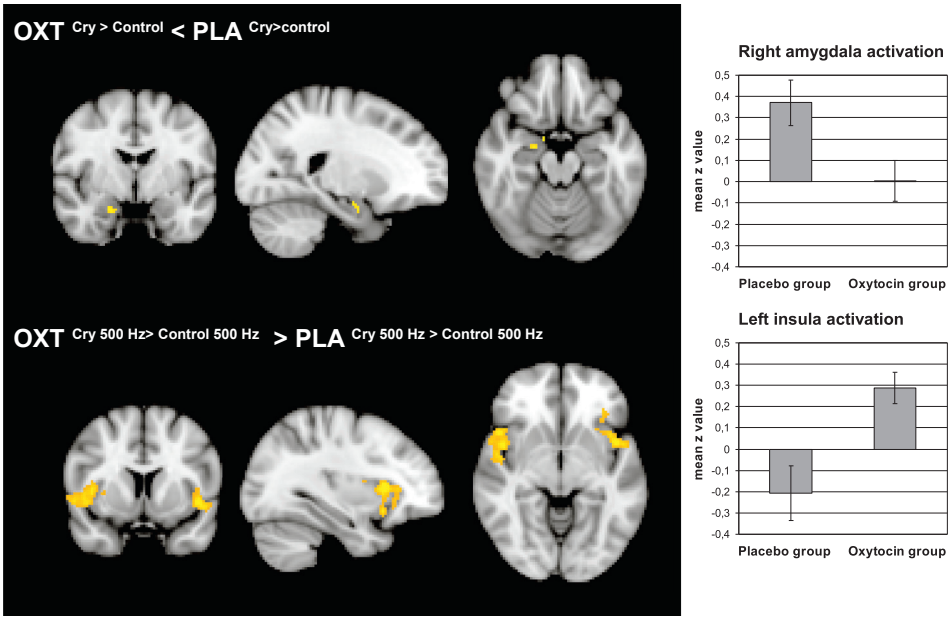

Figure 3. Top panel: oxytocin effect on right amygdala activation and mean *Z* values and standard errors for the oxytocin and placebo group for the contrast Cry combined > Control combined in the amygdala. Lower panel: oxytocin effect on bilateral insula and inferior frontal gyrus pars triangularis activation and mean *Z* values and standard errors for the oxytocin and placebo group for the contrast Cry 500 > Control 500 in the left insula. Both statistical images were thresholded using clusters determined by $Z > 2.3$ and a cluster corrected significance threshold of $p < 0.05$. The amygdala activation was revealed by ROI analysis limited to this search region, applying the same statistical threshold, however correcting only for the size of the ROI volume.

right amygdala when they listened to infant crying (see Table 1 and top panel Fig. 3). This contrast did not reveal significant activation in the other regions of interest.

We also examined the effect of oxytocin in the 500 Hz condition (Oxytocin $^{\text{Cry 500 Hz > Control 500 Hz}}$ > Placebo $^{\text{Cry 500 Hz > Control 500 Hz}}$). Whole brain analysis indicated that oxytocin increased bilateral insula and inferior frontal gyrus pars triangularis activation (Harvard–Oxford cortical atlas: frontal operculum) (see lower panel Fig. 3 and 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

Table 1. MNI coordinates, cluster size and Z-max values for significantly activated clusters.

Experimental effect	MNI coordinates			Cluster size	Peak Z
Region	x	y	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 x Drug effects					
OXT $^{\text{Cry > Control}}$ < PLA $^{\text{Cry > Control}}$					
R Amygdala	26	-12	-14	58	3.28[a]
OXT $^{\text{Cry 500 Hz > Control 500 Hz}}$ > PLA $^{\text{Cry 500 Hz > Control 500 Hz}}$					
R Planum polare	-52	-8	0	780	3.93
L Inferior frontal gyrus	50	16	-4	747	3.97

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

[a] ROI analysis, $p < 0.05$, corrected by cluster threshold ($z > 2.3$)

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, neither at whole group level (placebo and oxytocin), nor 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 in order to examine whether advanced age influenced the effects of oxytocin (Al-Azzawi & Palacios, 2009). 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 ANOVAs 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 by group interaction effects on any of the mood items anger ($F(1,40) = 0.02$, $p = .89$), frustration ($F(1,40) = 0.25$, $p = .62$), anxiety ($F(1,40) = 3.07$, $p = .09$), empathy ($F(1,40) = 0.03$, $p = .86$), happiness ($F(1,40) = 1.08$, $p = .30$) and calmness ($F(1,40) = 0.40$, $p = .53$). 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 to control sounds. Several studies have shown that the amygdala is involved in the perception of infant stimuli (Bos et al., 2010; Seifritz et al., 2003; Swain et al., 2008) and that oxytocin reduces amygdala activation during the perception of fear-inducing social stimuli (Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008). 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 (Heinrichs et al., 2001; Heinrichs et al., 2002).

Whereas 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 (Strathearn, Fonagy, Amico, & Montague, 2009) and the inferior frontal gyrus is important for affective prosodic comprehension (Leitman et al., 2010). Empathy is an important

prerequisite of parental sensitivity, defined as parents' ability to perceive child signals, to interpret these signals correctly, and to respond to them promptly and appropriately (Ainsworth, Blehar, Waters, & Wall, 1978). Indeed, empathy has been found related to parental responsiveness to infant signals (Feldman, 2007; Gondoli & Silverberg, 1997; Leerkes, 2010). 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 others' emotions (Gallese, Keysers, & Rizzolatti, 2004; Iacoboni, 2009). The insula and inferior frontal gyrus are suggested to play an important role in this simulation process (Chakrabarti, Bullmore, & Baron-Cohen, 2006; Decety & Jackson, 2004; Jabbi, Swart, & Keysers, 2007), and oxytocin may 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. (2010) who found that testosterone increases insula activation in response to infant crying, possibly by influencing the oxytonergic system. Testosterone has been linked to oxytocin as it is metabolized into estradiol which subsequently influences oxytocin levels (Choleris, Devidze, Kavaliers, & Pfaff, 2008; Cornil, Ball, & Balthazart, 2006).

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 to control sounds. These regions are important for language processing and social perception (Baron-Cohen et al., 1999; Leitman et al., 2010). Previous studies have also shown involvement of the superior temporal gyrus and the fusiform gyrus on neural responses to infant stimuli (Strathearn et al., 2009; Swain et al., 2008). In addition, we found increased activation in the amygdala during infant crying compared to control sounds. Amygdala activation was restricted to the right hemisphere. This is in line with previous studies of responsiveness to infant crying (Bos et al., 2010; Lorberbaum et al., 2002; Seifritz et al., 2003). It has been suggested that this right hemisphere dominance has evolved as most women carry their infant on their left arm (Harris, Cardenas, Spradlin, & Almerigi, 2010; Reissland, Hopkins, Helms, & Williams, 2009; Sieratzki & Woll, 1996).

In contrast with our expectations the thalamus did not show significantly increased activation during exposure to infant crying, and no different activation in the oxytocin as compared to 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 (Bos et al., 2010; Lorberbaum et al., 2002; Swain et al., 2008), and other studies not finding significant thalamic activation to infant cues (Seifritz et al., 2003; Strathearn et al., 2009). These equivocal results might be explained by different characteristics of cry and control stimuli. For instance, in some studies mothers listened to their own infant's crying whereas other studies used cry sounds of unknown infants. Swain et al. (2008) showed that, among other regions, the thalamus was significantly more active when mothers listened to their own infant's cry compared to 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 (Beery, Lacey, & Francis,

2008; Campbell, Ophir, & Phelps, 2009), but the number of studies reporting oxytocin effects on thalamic activation is limited (e.g. Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008). 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 (Altemus, Roca, Galliven, Romanos, & Deuster, 2001; Liedman et al., 2008; Stock, Bremme, & Uvnasmoberg, 1991), 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, the majority of the participants used oral contraceptives dampening menstrual cycle-related hormonal fluctuations (Salonia et al., 2005). Furthermore, our findings of reduced amygdala activation and increased insula activation were not influenced by a general change in mood since oxytocin did not affect self-reported emotional states. These results converge with several other studies that reported no effects of oxytocin on emotional state (Kirsch et al., 2005; Naber, Van IJzendoorn, Deschamps, Van Engeland, & Bakermans-Kranenburg, 2010).

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 may have influenced the results. However, the majority of our participants were mono- and dizygotic twin pairs, perfectly matched on age and global child rearing experiences, and even on genotype in monozygotic 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 (De Dreu et al., 2010). Domes et al. (2010) found increased amygdala activation in females in the oxytocin condition in response to fearful or angry adult faces. This finding seems in contrast with the previously reported effects of oxytocin found in men, but it might reflect enhanced vigilance to threat signals evolved in order to protect the child, thus triggering an aggressive reaction to frightening faces of adult strangers (De Dreu et al., 2010). In an ideal future study the Domes et al. (2010) 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 (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Naber et al., 2010). 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 may trigger child abuse or neglect (Reijneveld et al., 2004). However, not all parents with irritable infants become abusive, and differences in oxytocin levels may 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.

3 No laughing matter: Intranasal oxytocin administration changes functional brain connectivity during exposure to infant laughter

Madelon M.E. Riem, Marinus H. van IJzendoorn, Mattie Tops, Maarten A.S. Boksem, Serge A.R.B. Rombouts, & Marian J. Bakermans-Kranenburg (2012). Neuropsychopharmacology, 37, 1257-1266.

Abstract

Infant laughter is a rewarding experience. It activates neural reward circuits and promotes parental proximity and care, thus facilitating parent-infant attachment. The neuropeptide oxytocin might enhance the incentive salience of infant laughter by modulating neural circuits related to the perception of infant cues. In a randomized controlled trial with functional magnetic resonance imaging we investigated the influence of intranasally administered oxytocin on functional brain connectivity in response to infant laughter. Blood oxygenation level-dependent responses to infant laughter were measured in 22 nulliparous women who were administered oxytocin and 20 nulliparous women who were administered a placebo. Elevated oxytocin levels reduced activation in the amygdala during infant laughter and enhanced functional connectivity between the amygdala and the orbitofrontal cortex, the anterior cingulate, the hippocampus, the precuneus, the supramarginal gyri and the middle temporal gyrus. Increased functional connectivity between the amygdala and regions involved in emotion regulation may reduce negative emotional arousal while enhancing the incentive salience of the infant laughter.

Introduction

The laugh of an infant is a uniquely rewarding experience for parents. It provokes feelings of love and happiness and promotes infant survival by eliciting parental proximity and care (Bowlby, 1969/1982; Groh & Roisman, 2009; Mendes, Seidl-de-Moura, & Siqueira, 2009). Laughter is suggested to be the outcome of a long evolutionary history (Van Hooff, 1972), and its production as well as perception might be hardwired in human beings (Owren & Bachorowski, 2003). Whereas infant smiling is one of the basic attachment behaviors that create closer proximity to a protective caregiver (Bowlby, 1969/1982; Sroufe & Waters, 1976) infant laughter is most easily released by tickling and other forms of rough-and-tumble play (Sroufe & Waters, 1976). The infant's laughter in its turn may activate neural reward centers in the parental brain (Kringelbach, 2005; Kringelbach et al., 2008; Strathearn, Fonagy, Amico, & Montague, 2009) and reinforce parental playful interactions. Oxytocin is a neuropeptide that facilitates the onset of maternal behavior and mother-infant attachment (Carter, 1998; Galbally, Lewis, Van IJzendoorn, & Permezel, 2011; Insel, 2010) and is involved in the perception of infant vocalizations (Riem et al., 2011; Riem, Pieper, Out, Bakermans-Kranenburg, & Van IJzendoorn, 2011). Oxytocin might enhance the incentive salience of infant laughter by modulating neural circuits related to the perception of infant cues and thus motivating sensitive responsiveness to infant laughter. To our knowledge, this is the first randomized controlled study to examine the effects of intranasally administered oxytocin on functional brain connectivity in response to infant laughter.

Oxytocin administration studies have shown that it stimulates a range of social behaviors (for a meta-analysis, see Van IJzendoorn and Bakermans-Kranenburg, 2012) including empathy (Bartz, Zaki, Bolger, et al., 2010), mind-reading (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007), trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), in-group altruism (De Dreu et al., 2010) and paternal stimulating play (Naber, Van IJzendoorn, Deschamps, Van Engeland, & Bakermans-Kranenburg, 2010). Feldman, Weller, Zagoory-Sharon, and Levine (2007) showed that maternal oxytocin levels across pregnancy are predictive of higher quality of postpartum maternal behavior. Oxytocin has anxiolytic and stress-reducing effects in breastfeeding mothers (Heinrichs et al., 2001; McCarthy, McDonald, Brooks, & Goldman, 1996) and this might increase mothers' sensitivity to infant signals including infant crying but also infant smiling and laughing. Taylor also suggested that oxytocin modulates stress responses and is implicated in the seeking of affiliative contact in response to stress (Taylor & Samson, 2005; Taylor, 2006). Although infant crying is very different from infant laughter, oxytocin seems to enhance sensitivity to both infant signals. For example, Strathearn et al. (2009) found that mothers with a strong increase in peripheral oxytocin release while interacting with their infants show more activation in neural reward systems such as the OFC and the ventral striatum during the perception of their smiling infant than mothers with lower oxytocin levels. This finding might indicate that oxytocin increases the 'incentive salience' of infant laughter (Berridge, 2007).

One important target of oxytocin is the amygdala (Meyer-Lindenberg, Domes, Kirsch, & Heinrichs, 2011), a neural structure that is part of the neural network involved in emotional processing. In a previous study we found that oxytocin reduced amygdala responses to infant crying whereas it increased activation of the insula and inferior frontal gyrus (IFG) (Riem et al., 2011), brain regions important for empathy, emotion understanding and maternal bonding (Bartels & Zeki, 2004; Shamay-Tsoory, 2011). The amygdala is also referred to as a neural hub because of its high degree of connectivity, which is critical for the flow and integration of information between regions (Pessoa, 2008). It is strongly connected with other brain regions involved in emotional processing such as the orbitofrontal cortex (OFC), the supra- and subgenual parts of the anterior cingulate cortex (ACC), the brainstem, and the thalamus (Bos, Panksepp, Bluthé, & Honk, 2012; Pessoa, 2008).

Several studies have shown that by modulating amygdala activity hormones can shift neural output towards other brain regions within this network. For example, Kirsch et al. (2005) showed that oxytocin reduces amygdala-brainstem coupling that is important for fear and arousal. Van Wingen, Mattern, Verkes, Buitelaar, and Fernandez (2010) showed that OFC-amygdala coupling was reduced after testosterone administration. The OFC is involved in reward and hedonic processing and it exhibits a specific neural response to infant stimuli (Kringelbach, 2005; Kringelbach et al., 2008; Noriuchi, Kikuchi, & Senoo, 2008). Furthermore, previous studies have shown that the posterior OFC and ACC are involved in the perception of infant crying (Laurent & Ablow, 2011; Swain et al., 2008). Whereas most studies focused on infant crying only, Seifritz et al. (2003) showed that the ACC was deactivated during both infant laughter and crying, indicating its important role in parent-infant interaction. Both structures are important for emotional regulation, in particular for the reduction of anxiety, by their inhibitory influence on the amygdala (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Hahn et al., 2011; Meyer-Lindenberg et al., 2011; Stein et al., 2007; Swain et al., 2008). Thus, oxytocin might stimulate mother-infant bonding by modulating connectivity between the amygdala, ACC and OFC and thereby enhancing the regulation of negative emotions and the experience of reward during mother-infant interaction (Bos et al., 2012).

In this study, we examined the influence of intranasally administered oxytocin on neural responses to infant laughter with functional magnetic resonance imaging (fMRI). Whole brain analysis was performed to explore the neural effects of oxytocin on functional activation during infant laughter. We focus our analyses on functional activation of the insula, IFG and amygdala as a prior study showed that oxytocin modulated activation in these regions during the perception of infant crying (Riem et al., 2011). Moreover, previous fMRI studies indicated that the insula and amygdala are involved in the perception of infant and adult laughter (Sander, Brechmann, & Scheich, 2003; Sander, Frome, & Scheich, 2007; Sander & Scheich, 2001, 2005; Seifritz et al., 2003). Furthermore, we examined oxytocin effects on the ventral striatum, the ACC, and the OFC because of the suggested significance of these regions in mother-infant bonding and reward processing (Berridge & Kringelbach, 2008; Kringelbach, 2005;

Kringelbach et al., 2008; Seifritz et al., 2003; Strathearn et al., 2009).We expected that oxytocin administration would be related to increased activity in the ventral striatum, ACC, OFC, insula and IFG and decreased activity in the amygdala. In addition, with psychophysiological-interaction (PPI) analysis we examined whether oxytocin affects amygdala-connectivity during the perception of infant laughter. In region of interest (ROI) analyses we tested whether oxytocin modulated functional connectivity between the amygdala, OFC and ACC during infant laughter.

Method

Participants

Participants were selected from a larger study investigating caregiving responses and physiological reactivity to infant crying (Out, Pieper, Bakermans-Kranenburg, & Van IJzendoorn, 2010). The original sample consisted of 50 male and 134 female adult twin pairs. Zygosity was determined on the basis of a zygosity questionnaire (Magnus, Berg, & Nance, 1983) and additional genetic analysis of *six* polymorphisms; results indicated that 12 twin pairs (6.5% of the sample) classified as monozygotic (MZ) on the basis of the questionnaire were in fact dizygotic (DZ). A group of 44 right-handed females were recruited, 22 from MZ twin pairs and 22 from DZ twin pairs, without children of their own, in good health, without hearing impairments and MRI contraindications, pregnancy, psychiatric or neurological disorders, and screened for alcohol and drug use. A between subject-design with twin siblings was used in order to avoid time effects (e.g. decreased neural responses to infant laughing due to habituation) and to minimize pre-existing differences (e.g. age, child-rearing experiences, genetics) between the oxytocin and placebo group. Two monozygotic siblings were excluded from the analyses due to excessive head movement during fMRI scanning (peak displacement = 4 mm). Twin siblings of 10 participants did not participate due to MRI contraindications or other exclusion criteria, resulting in a sample of 30 participants from twin pairs (8 MZ, 7 DZ) and 12 participants without twin sibling (4 MZ, 8 DZ). The mean age of the participants was 28.71 years (*SD* =6.93, 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. During the luteal phase, plasma oxytocin levels are lower (Salonia et al., 2005) and more responsive to stimulation such as by nipple stimulation (Leake, Buster, & Fisher, 1984). Therefore, effects of oxytocin nasal administration might be more pronounced during the luteal phase. Approximately forty minutes before the start of the fMRI data acquisition subjects took nasal spray containing oxytocin or placebo. Time between oxytocin/placebo administration and data acquisition was similar to previous fMRI studies (Marsh,

Yu, Pine, & Blair, 2010; Riem et al., 2011; Rimmele, Hediger, Heinrichs, & Klaver, 2009). 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. Participants were instructed to attend to the sounds they would hear. Before drug administration and after fMRI scanning participants completed a mood questionnaire in order to track mood changes following drug administration. Participants rated on 7-point Likert scales how much anger, sadness, pleasantness, empathy, happiness, warmth and calmness they felt. In addition, after fMRI scanning participants rated how healthy the infant laughter sounded, and how much warmth and affection they felt while listening to the laughing sounds. Furthermore, participants rated whether they felt irritated while listening to the control stimuli on 5-point Likert scale (1 = not irritated, 5 = irritated).

Experimental paradigm

Participants listened to intensity-matched infant crying and infant laughter sounds with a duration of 6 seconds (samples 261 and 110 of the International Affective Digitized Sounds (IADS) system, Bradley & Lang, 1999) . These infant sounds have been used in a previous fMRI study (Seifritz et al., 2003). Neutral auditory control stimuli were created identical to the original auditory stimuli in terms of duration, intensity, spectral content, and amplitude envelope, but lacking recognizable qualities. The infant sounds and control sounds were presented in 8 cycles, each cycle consisting of 4 sounds (Cry, Cry-control, Laughter, Laughter-control). The order of presentation of sounds within each cycle was random; the intertrial-interval was 6 s. In this study we focus only on neural responses to infant laughter. Elsewhere we report on the functional activation during the perception of (a different set of) infant crying sounds (Riem et al., 2011).

Oxytocin versus placebo

One sibling from each twin pair 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. Participants without a twin sibling were also randomly assigned to the oxytocin and placebo condition. Because no twin pair was assigned to either the experimental or the placebo condition, data concerning the effects of the experimental administration of oxytocin were statistically independent. The use of twins randomized across conditions enhanced comparability of the groups in areas other than the experimental manipulation. Approximately forty 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. Menstrual phase and use of oral contraceptives were balanced across the placebo and oxytocin group: 11 participants in the oxytocin and 11 participants in the placebo group were in the luteal phase, whereas 8 participants in the oxytocin group and 9 participants in the placebo group were in the follicular phase. 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.

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, voxelsize .875×.875×1.2 mm). In addition, a high-resolution EPI scan was obtained (for registration purposes) (TR = 2.2 sec; TE = 30 msec, flip angle = 80°, 84 slices, voxelsize 1.96×1.96×2.00 mm. For fMRI, a total of 185 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)). 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 using FSL version 5.98 (FMRIB's Software Library, www.FMRIb.ox.ac.uk/fsl, (Smith et al., 2004). The following pre-statistics processing was applied: motion correction (Jenkinson, Bannister, Brady, & Smith, 2002), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 8.0 mm, and high-pass temporal filtering (highpass filter cutoff = 100.0s). Functional scans were registered to the high-resolution EPI-images, which were registered to the T1-weighted images, which were registered to standard space (Jenkinson et al., 2002; Jenkinson & Smith, 2001).

In native space, functional activation was examined using general linear model analysis. Each sound (Cry, Cry-control, Laughter, Laughter-control) was modeled separately as a square-wave function. Each predictor was then convolved with a double gamma hemodynamic response function and its temporal derivative was added to the model, giving 8 regressors. We assessed the contrast Laugh > Laugh-control in order to identify regions involved in the perception of infant laughing.

Second, we examined psychophysiological interactions (PPI), i.e. condition-dependent changes in the covariation of the response between a seed region and other brain regions (Friston et al., 1997). We used the left and right amygdala as seed regions because we were primarily interested in the modulation effects of oxytocin on functional connectivity with the amygdala. We extracted the mean time series for each participant from the left and the right amygdala, defined using the Harvard-Oxford subcortical atlas. These times series were then used as a physiological regressor in the model. We applied two separate models: one to analyze left amygdala connectivity, and one to study right amygdala connectivity. A contrast between Laughter and Laughter-control (Laugh>Laugh-control) was created and used as psychological regressor. This regressor was convolved with a double gamma hemodynamic response function and its temporal derivative was

added to the model. Furthermore, the Cry sound and the Cry-control sound were included in the model as two separate regressors, both convolved with a double gamma hemodynamic response function. The temporal derivatives of these two regressors were also added to the model. Finally, the interaction between the psychological regressor and the time series from the left or right amygdala was modeled, giving 8 regressors. We assessed the positive and negative contrast of the interaction in order to examine condition-dependent changes in functional connectivity.

All first-level contrast images (Laughter > Control and PPI) and the corresponding variance images were transformed to standard space and submitted to second-level mixed-effects group whole brain analyses. For functional activation and PPI analysis, group means were tested using one-sample t-tests and we tested for group differences using two-sample t-tests on these contrasts 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 the analyses of the group means and group differences in the functional activation and PPI analysis. The statistical images were thresholded using clusters determined by $Z > 2.3$ and a cluster corrected significance threshold of $p<.05$ (Worsley, 2001).

In addition to the whole brain analyses, regions of interest (ROI) analyses were performed in FSL to investigate changes in activation of a priori specified regions. For the perception of infant vocalizations these regions are the amygdala, ventral striatum/nucleus accumbens, inferior frontal gyrus and the insula (Bos et al., 2012; Riem et al., 2011). These were defined using the Harvard–Oxford (sub) cortical atlas (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). ROI analyses were limited to these search regions, applying the same statistical threshold as for the whole brain analyses, but correcting only for the size of ROI volumes. For PPI, ROIs were the anterior cingulate and the orbitofrontal cortex, again defined with the Harvard–Oxford cortical atlas. ROI analyses were only conducted when these regions were not significantly activated in the whole brain analysis. The selection of these ROIs was based on a neural model for the effects of neuropeptides on brain connectivity (Bos et al., 2012). The amygdala is strongly connected to the medial and posterior regions of the orbitofrontal and the rostral and caudal regions of the anterior cingulate cortex, and it has been suggested that oxytocin shifts neural output towards these brain regions by modulating amygdala activity (Amaral & Price, 1984; Bos et al., 2012; Carmichael & Price, 1995; Kringelbach & Rolls, 2004). Mean Z values for significantly activated regions were calculated using Featquery (http://www.fmrib.ox.ac.uk/fsl/feat5/featquery.html) for visualization purposes in figures.

Results

To examine whether oxytocin affected neural responses to infant laughter we contrasted the oxytocin group with the placebo group (Oxytocin $^{\text{Laugh > Control}}$ > Placebo $^{\text{Laugh > Control}}$ and Oxytocin $^{\text{Laugh > Control}}$ < Placebo $^{\text{Laugh > Control}}$). There were no

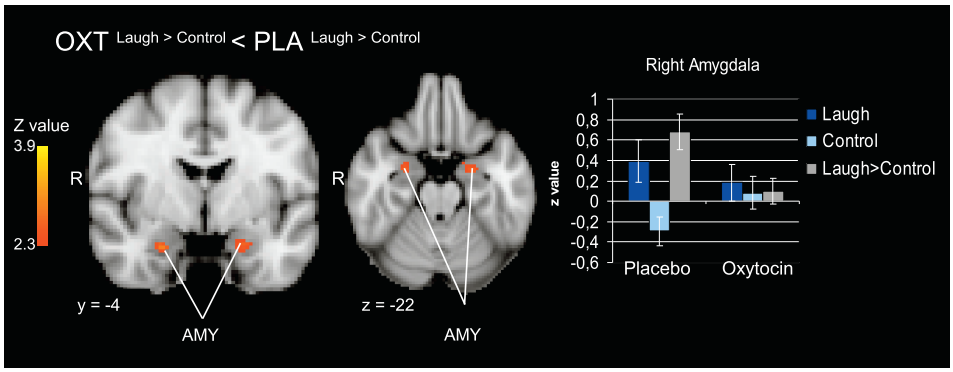

Figure 1. Oxytocin effect on bilateral amygdala (AMY) activation and mean *Z* values and standard errors of right amygdala activation during Laugh, Laugh-control and Laugh>Control in the oxytocin and placebo group. Region of interest analysis, $p<.05$, corrected by cluster threshold ($Z > 2.3$).

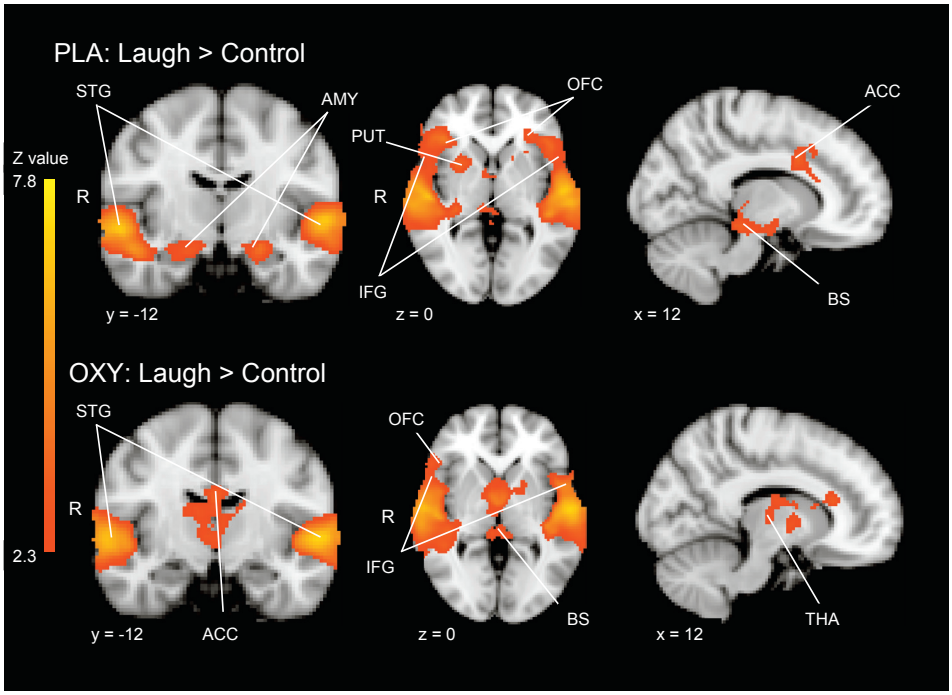

Figure 2. Top panel: Significant activation in bilateral temporal poles, the bilateral superior temporal gyrus (STG), the bilateral orbitofrontal cortex (OFC), the bilateral inferior and superior frontal gyrus (IFG), the bilateral amygdala (AMY), the brainstem (BS), the anterior cingulate cortex (ACC) and the right putamen (PUT) for the contrast Laugh > Control in the placebo group. Lower panel: Significant activation in bilateral temporal poles, the bilateral superior temporal gyrus, the right orbitofrontal cortex, the bilateral inferior frontal gyrus, the brainstem, the bilateral thalamus (THA) and the anterior cingulate for the contrast Laugh > Control in the oxytocin group. Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p< .05$.

significant group differences in the whole brain analysis. ROI analysis showed that, compared to the placebo group, participants who received oxytocin showed reduced activation in the amygdala when they listened to infant laughter compared with control sounds. There was one significant cluster in the left amygdala and one significant cluster in the right amygdala (cluster 1: size = 54, peak *Z* = 2.79, MNI coordinates x,y,z (mm) = -22, -10, -16, cluster 2: size = 22, peak *Z* = 2.87, MNI coordinates x,y,z (mm) = 24, -4, -24) (Figure 1). There were no significant effects of oxytocin in the other regions of interest.

In the whole brain analysis of functional activation the contrast of infant laughter versus control sound revealed two large clusters of activation in the placebo group with peak voxels in the superior temporal gyri (Cluster 1: size = 10,831 voxels, peak *Z* = 6.44, MNI coordinates x,y,z (mm) = 56,-14,-4, Cluster 2: size = 7,295 voxels, peak *Z* = 6.30, MNI coordinates x,y,z (mm) = -58,-12,0, see Table 1 for an overview of statistics of the local maxima within these clusters). The pattern of activation included the bilateral temporal poles, the bilateral superior temporal gyrus, the bilateral orbitofrontal cortex, the bilateral inferior and superior frontal gyrus, the bilateral amygdala, the brainstem and the right putamen (see top panel Figure 2 for functional activation in the placebo group and lower panel Figure 2 for functional activation in the oxytocin group). ROI analyses indicated that there was no significant activation in the ventral striatum or in the insula during infant laughter compared with control sounds.

Table 1. MNI coordinates and Z-max values for local maxima within the significantly activated clusters during exposure to infant laughter compared to control sounds in the placebo group.

			MNI coordinates		
Cluster	Region	*Z*	x	y	z
2	R Superior Temporal Gyrus	6.44	56	-14	-4
2	R Superior Temporal Gyrus	5.45	66	-24	6
2	R Superior Temporal Gyrus	5.42	50	-4	-18
2	R Middle Temporal Gyrus	4.92	50	-12	-16
2	R Middle Temporal Gyrus	4.55	62	-46	12
2	R Precentral Gyrus	4.41	60	-2	46
1	L Superior Temporal Gyrus	6.30	-58	-12	0
1	L Superior Temporal Gyrus	6.12	-62	-24	4
1	L Planum Temporale	5.68	-50	-34	8
1	L Planum Temporale	5.49	-48	-32	4
1	L Superior Temporal Gyrus	4.88	-66	-38	8
1	L Supramarginal Gyrus	4.32	-64	-42	18

In the next step we performed PPI analyses to examine whether oxytocin affected functional connectivity with the amygdala when participants listened to infant laughter compared to control sounds. The whole brain analysis revealed that oxytocin significantly enhanced connectivity between the right amygdala and the left orbitofrontal cortex, the bilateral hippocampus, the left precuneus, the

right angular gyrus, and the right middle temporal gyrus during infant laughter compared to the control sound (see Table 2 and Figure 3). ROI analyses showed that functional connectivity between the bilateral amygdala and the caudal anterior cingulate was also enhanced by oxytocin during the exposure to infant laughter compared to control sounds (see Figure 3 and Table 2 for an overview of functional connectivity in the oxytocin and placebo group). The average Laugh > Control connectivity change (or PPI) in the ACC in the oxytocin group was positive, but not significant (peak voxel oxytocin > placebo comparison x,y,z (mm) = -6, 10, 38, Z = 2.30), while it is significantly increased as compared to the placebo PPI (peak voxel oxytocin > placebo comparison x,y,z (mm) = -6, 10, 38, Z = -2.56). Thus, there was a significant group difference although the PPI was not significant in the oxytocin group. We performed additional analyses examining whether left amygdala connectivity with the ACC was significantly different from right amygdala connectivity with the ACC. There was no significant difference (F(1,40) = 0.19, p = .89) and no significant hemisphere x treatment group (oxytocin vs placebo) interaction (F(1,40) = 1.07, p = .31). In sum, oxytocin decreased amygdala activation relative to the placebo condition during exposure to infant laughter and increased functional coupling between the amygdala and regions implicated in the perception and regulation of emotional cues.

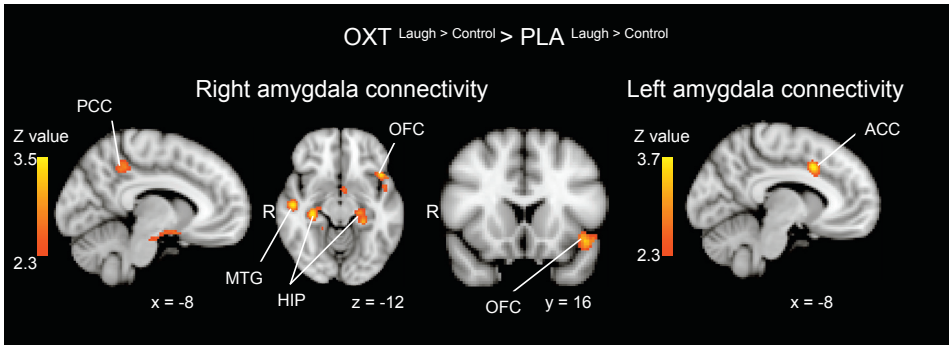

Figure 3. Left panel: Oxytocin induced stronger functional connectivity between the right amygdala and the hippocampus (HIP), precuneus (PCC), orbitofrontal cortex (OFC) and middle temporal gyrus (MTG) during the perception of infant laughter compared to control sound. Right panel: Oxytocin also enhanced functional connectivity between the left amygdala and the anterior cingulate cortex (ACC) during the perception of infant laughter compared to control sound. Statistical images were thresholded with clusters determined by Z > 2.3 and a cluster-corrected significance threshold of p< .05.

Independent-sample t-tests were used to examine the subjective rating of the laughing and control sounds. Participants who received oxytocin experienced more warm feelings (M = 6.14, SD = 1.11) when listening to the laughing sounds compared to participants in the placebo group (M = 5.25, SD = 1.62), t(39) = 2.07, p < .05 (not significant after Bonferroni correction for multiple comparisons). However, there was no significant group difference in reported affection, t(39) = 1.14, p = .26 and no significant group difference in how healthy the participants rated the laughing sound t(39) = 0.49, p = .63. Participants did not feel much

irritation while listening to the control sounds ($M = 2.05$, $SD = 1.32$) and there was no significant group difference between the oxytocin and placebo group $t(39) = 0.94$, $p = .35$. To control for nonspecific effects of oxytocin on self-reported mood, 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 x group interaction effects on any of the mood items: anger $F(1,40) = 0.07$, $p = .80$, sadness $F(1,40) = 0.22$, $p = .64$, pleasantness $F(1,40) = 0.15$, $p = .90$, empathy $F(1,40) = 0.00$, $p = .99$, happiness $F(1,40) = 2.69$, $p = .11$, warm feeling $F(1,40) = 0.36$, $p = .55$, and calmness $F(1,40) = 0.01$, $p = .91$.

Discussion

Our study demonstrates that oxytocin reduces amygdala activation relative to the placebo condition when individuals listen to infant laughter compared with control sounds. Oxytocin has stress-reducing effects in lactating mothers (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Heinrichs et al., 2001) which might enable them to be more sensitive to infant cues. Recent evidence indicates that inhibition of the amygdala, a brain region involved in anxiety and emotional arousal (LeDoux, 2000; Morrison & Salzman, 2010), might be the underlying neural mechanism of these calming oxytocin effects (Gamer, Zurowski, & Buchel, 2010; Kirsch et al., 2005; Riem et al., 2011). In addition, we found that oxytocin increased functional connectivity between the amygdala and neural reward regions, the OFC and the caudal ACC (Berridge & Kringelbach, 2008; Haber & Knutson, 2010; Kringelbach, 2005). Our findings provide empirical support for the neural model on the effects of neuropeptides on brain connectivity proposed by Bos et al. (2012). Increased functional connectivity between the OFC, ACC and amygdala may promote mother-infant attachment by enhancing cognitive control over negative emotionality and at the same time increasing the incentive salience of infant laughter (Berridge, 2007; Berridge & Kringelbach, 2008; Bos et al., 2012).

Infant laughter is easily released during playful interactions with their parents which are highly rewarding for both parent and infant (Feldman, 2003; Sroufe & Waters, 1976). Previous studies on oxytocin and parenting showed that oxytocin promotes such playful interactions. For example, Feldman et al. (2010) showed that fathers with high levels of oxytocin displayed more stimulatory contact during play with their child. In a complementary study, Naber et al. (2010) found that intranasally administered oxytocin enhances paternal playful interaction. The current study is the first to examine the neural mechanism underlying the effect of oxytocin on the perception of playful infant vocalizations. Future studies should examine the neural mechanisms underlying the rewarding effects of playful parent-infant interactions in a more real-life situation, for example by studying neural activation and connectivity during the presentation of video fragments of parent-infant playful interactions.

Furthermore, we found that oxytocin increased functional connectivity between the amygdala and the hippocampus, middle temporal gyrus and precuneus

Table 2. Overview of functional connectivity during infant laughter compared to control sound: MNI coordinates, cluster size, and *Z*-max values for significantly clusters of functional connectivity.

Seed Region	Experimental effect	Functional connectivity	MNI coordinates			Cluster size	Peak Z
			x	y	z		
R Amygdala	$PLA^{Laugh < Control}$	R Intracalcarine Cortex	6	-68	16	237	2.95
	$OXY^{Laugh > Control}$	R Brainstem	4	-6	-20	479	3.38
		L Orbitofrontal cortex	-46	18	-12	436	3.28
		R Supramarginal Gyrus	52	-40	34	210	3.30
	$OXY^{Laugh < Control}$	R Occipital Pole	32	-94	-10	398	3.35
	$OXY^{Laugh>Control}>PLA^{Laugh>Control}$	L Hippocampus	-16	-14	-18	549	3.13
		L Precuneus	-16	-44	42	266	3.16
		R Middle Temporal Gyrus	52	-16	-14	233	3.51
		R Parahippocampal Gyrus	30	-26	-12	201	3.37
		L Orbitofrontal Cortex	-44	18	-12	185	3.32
		L Angular Gyrus	-62	-60	26	184	3.24
		L Anterior Cingulate	-6	10	38	116	3.33[a]
	$OXY^{Laugh>Control}<PLA^{Laugh>Control}$	R Lateral Occipital Cortex	18	-86	20	177	3.15
L Amygdala	$PLA^{Laugh < Control}$	R Orbitofrontal Cortex	34	32	-8	101	3.46[a]
	$OXY^{Laugh > Control}$	L Anterior Cingulate	-8	12	36	120	3.33[a]
	$OXY^{Laugh < Control}$	R Middle Temporal Gyrus	66	-56	-4	491	3.02
		R Occipital Pole	24	-90	2	358	3.42
		R Inferior Temporal Gyrus	58	-36	-18	233	3.23
	$OXY^{Laugh>Control}>PLA^{Laugh>Control}$	L Anterior Cingulate	-8	10	38	172	3.70[a]
	$OXY^{Laugh>Control}<PLA^{Laugh>Control}$	R Lateral Occipital Cortex	20	-86	18	705	3.32

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

during infant laughter compared to control sound. Although in previous studies the hippocampus has not been directly implicated in parental care, it is known to be affected by parenting experiences, possibly via the altered parental hormonal levels after child birth (Leuner, Glasper, & Gould, 2010). Several studies indicate that amygdala-hippocampus interactions are crucial for emotional memory (Schaefer & Gray, 2007), an important factor in parenting that can be enhanced by oxytocin (Bartz, Zaki, Ochsner, et al., 2010; Guastella, Mitchell, & Mathews, 2008; Rimmele et al., 2009). The middle temporal gyrus and precuneus are part of a network involved in the perception of speech and prosody (Leitman et al., 2010; Price, 2010; Turken & Dronkers, 2011) and in aspects of social cognition such as mentalizing and emotion understanding (Atique, Erb, Gharabaghi, Grodd, & Anders, 2011; Leitman et al., 2010). Pessoa (2008) suggested that the high degree of connectivity between the amygdala and other regions involved in emotional processing might serve the integration of emotion and cognition and the evaluation of sensory information. Oxytocin might facilitate evaluation of and responding to emotional stimuli by modulating neural connectivity (Pessoa, 2008; Salzman & Fusi, 2010). This is supported by a study of Gamer et al. (2010) who showed that oxytocin increased functional coupling between the amygdala and the superior colliculus as well as gaze changes towards the eyes of an emotional stranger in order to facilitate the classification of the emotion.

In the placebo group, we found an increase in activation in the temporal poles, the orbitofrontal cortex, the inferior and superior frontal gyrus, the amygdala, the brainstem and the putamen in response to infant laughter compared to control sounds. Previous fMRI studies on the perception of infant stimuli also reported activation in these regions. For example, Strathearn et al. (2008) presented mothers with images of their own happy infants and found significant activation in the putamen, a subregion of the ventral striatum which is important for reward processing. In a previous study on infant crying we also found significant activation in the inferior frontal gyrus and the temporal poles (Riem et al., 2011), regions that are involved in theory of mind and empathy (Chakrabarti, Bullmore, & Baron-Cohen, 2006; Decety & Jackson, 2004). However, in contrast with our previous study, the insula was not significantly activated during exposure to infant laughter and oxytocin did not modulate activation in this region. Previous studies have shown that the insula is involved in feeling empathy for others, in particular when observing others in pain (Lamm, Decety, & Singer, 2011). For example, Lang et al. (2011) indicated that the insula was significantly more activated when listening to pain expressions compared to positive stimuli such as laughter, which is in line with our respective results for activation during exposure to infant laughter and infant crying.

One limitation of this study is that the use of 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. Second, it should be noted that neural responses to infant laughter might be affected by the infant crying sounds that were also presented during the experimental paradigm because the

infant laughter-crying contrast might have enhanced the rewarding experience of infant laughter. In addition, the physiological effects induced by intranasally administered oxytocin are not well-understood and might be different from the effects of endogenous oxytocin secretion. Furthermore, functional connectivity using fMRI is a correlation method that does not allow conclusions about the (direction of the) causal relation between the OFC, ACC and amygdala during exposure to infant laughter. Several studies suggest however that the OFC and ACC regulate negative emotionality by inhibiting the amygdala (Banks et al., 2007; Hahn et al., 2011; Stein et al., 2007; Swain et al., 2008). Furthermore, it should be noted that the regions in which we found significant connectivity changes in the oxytocin-placebo comparison did not all have a significant PPI in the oxytocin group. Finally, our findings can only be generalized to women without children. The perception of infant signals is influenced by parental status (Seifritz et al., 2003), possibly due to the altered oxytocinergic system after child birth, lactation and parent-infant contact (Feldman et al., 2010). More research is needed to examine oxytocin effects on functional activation and connectivity during infant signals in parents. Intranasal oxytocin effects might be even more pronounced in parents, because of the emotional and biological significance of their own infant's laughing and crying.

Our findings support neural models on functional connectivity between the amygdala, OFC and ACC proposed by Kringelbach (2005), Bos et al. (2012) and Meyer-Lindenberg et al. (2011). Kringelbach et al. (2008) showed that the medial OFC exhibits a very early and specific pattern of activity to infant cues. Therefore, it has been speculated that the medial OFC might be the neural base for the innate releasing mechanism described by Lorenz for affection and care of young infants (Lorenz, 1943). The OFC might encode rewarding characteristics of infant stimuli such as 'cuteness' that predispose parents to perceive infant stimuli as special and that elicit nurturing. The medial and posterior OFC, ACC and amygdala have strong reciprocal connections that are important for the effect of the encoded reward value on subsequent behavior, for example caregiving responses (Kringelbach, 2005). While the medial OFC and ACC have been suggested to be important hedonic hot spots of the brain (Berridge & Kringelbach, 2008; Kringelbach, 2005; Parsons, Young, Murray, Stein, & Kringelbach, 2010), they are also involved in emotion regulation by their inhibitory influence on the amygdala, especially the supra- and subgenual parts of the ACC (Banks et al., 2007; Hahn et al., 2011; Stein et al., 2007). Bos et al. (2012) suggested that oxytocin facilitates social bonding by enhancing cognitive control from prefrontal regions to regulate emotionality, as well as by its effects on the experience of reward during social interaction. Our findings are consistent with this model and suggest that oxytocin promotes parent-infant attachment by reducing negative emotional arousal while increasing the incentive salience of infant cues.

In conclusion, this is the first study to show the effects of oxytocin intranasal administration on functional activation and connectivity to infant laughter. We found that oxytocin decreases amygdala activation to infant laughter and increases functional connectivity between the amygdala and the OFC, ACC and several other brain regions involved in emotional processing. Our results extend

previous findings indicating a central role for oxytocin in parent-infant playful interactions and attachment formation (Feldman, 2003; Feldman et al., 2007; Naber et al., 2010). Increased functional connectivity between the amygdala, ACC and OFC may stimulate mother-infant bonding by enhancing the regulation of negative emotions and the experience of reward during parent-infant interaction. Thus, oxytocin increases the incentive salience of infant laughter, which might be one of the mechanisms that lead to enhanced sensitive responsiveness to infant cues and playful parent-infant interaction.

4 Attachment in the brain: Adult attachment representations predict amygdala and behavioral responses to infant crying

Madelon M.E. Riem, Marian J. Bakermans-Kranenburg, Marinus H. van IJzendoorn, Dorothée Out, & Serge A.R.B. Rombouts (2012). Attachment & Human Development, 14, 1261-6734.

Abstract

In the current study we demonstrate that adult attachment representations influence neural, emotional and behavioral responses to infant crying, thus validating the Berkeley Adult Attachment Interview with functional Magnetic Resonance Imaging. We examined amygdala activation, feelings of irritation, and the use of excessive force as indicated by grip strength using a hand-grip dynamometer during exposure to infant crying and scrambled control sounds in 21 women without children. Individuals with insecure attachment representations showed heightened amygdala activation when exposed to infant crying compared to individuals with secure attachment representations. In addition, insecure individuals experienced more irritation during infant crying and used more excessive force than individuals with a secure representation. Amygdala hyperactivity might be one of the mechanisms underlying the experience of negative emotions during exposure to infant crying in insecure individuals and might explain why insecure parents respond inconsistently to infant signals or reject their infants' attachment behavior.

Introduction

Infant crying is a salient attachment signal that contributes to infant survival through eliciting parental proximity and care (Bowlby, 1969/1982; Soltis, 2004). Crying evokes strong emotional reactions in parents, ranging from feelings of empathy to negative emotions such as anxiety and anger (Dix, 1991). Empathic emotional reactions motivate sensitive parental behaviors such as soothing or feeding the child, whereas negative emotional reactions increase the likelihood of using harsh responses that are aimed to stop the crying by all means because it is perceived as aversive (Dix, Gershoff, Meunier, & Miller, 2004). Not all parents are always able to respond in a sensitive way to their crying infant, and excessive, high-pitched crying can trigger even child abuse or neglect in some parents (Barr, Trent, & Cross, 2006; Out, Pieper, Bakermans-Kranenburg, Zeskind, & Van IJzendoorn, 2010; Reijneveld, Van der Wal, Brugman, Sing, & Verloove-Vanhorick, 2004; Soltis, 2004). Although it has been shown that adult attachment affects parental responding to infant crying (Leerkes & Siepak, 2006), little is known about the neural mechanisms underlying attachment-related individual differences in parenting behavior. The present study was designed to shed more light on the perception and processing of infant signals in individuals with different adult attachment representations by examining neural, emotional, and behavioral responses to infant crying.

Adult attachment reflects the current state of mind with respect to attachment and refers to the mental representation of past and present attachment experiences. Adult attachment can be measured with several instruments, but the 'gold standard' for the measurement of adult attachment is the Adult Attachment Interview (Hesse, 2008; George, Kaplan, & Main, 1985; Main & Goldwyn, 1984), a semi-structured interview in which participants are required to reflect upon their attachment-related experiences (Hesse, 2008; Main, Hesse, & Goldwyn, 2008). Participants are asked to describe their relationships with attachment figures, to give specific examples to support these descriptions and to evaluate their memories of attachment-related events from their current perspective. The coding system of the AAI includes three major adult attachment classifications: secure-autonomous (F), insecure-dismissing (Ds) and insecure-preoccupied (E) (see methods for descriptions of these classifications). The AAI has been administered to more than 10.000 respondents since its development (Bakermans-Kranenburg & Van IJzendoorn, 2009), and numerous studies support its validity and reliability (e.g., Bakermans-Kranenburg & Van IJzendoorn, 1993; Benoit & Parker, 1994; Crowell et al., 1996; Sagi et al., 1994).

Several studies have shown that individual differences in adult attachment, measured with the AAI, are related to different patterns of responding to infant signals and therefore affect infant attachment and developmental outcomes (see Van IJzendoorn, 1995). More specifically related to infant crying, secure-autonomous adults are suggested to be able to respond adequately to their crying infants since they are free of distorted perceptions of their infants' needs (Blehar, Waters, & Wall, 1978; see also Mesman, Oster, & Camras, 2012), whereas individuals with an insecure attachment representation may respond

inconsistently or reject their infants' attachment behavior. As a consequence, secure parents more often have infants who are securely attached, whereas parents with an insecure attachment representation tend to have insecurely attached infants (Main, 2000). Leerkes and Siepak (2006) examined attachment-related predictors of emotional and cognitive responses to infant distress. They found that adult attachment as measured with self-report questionnaires influenced how individuals perceived infant crying. Individuals with insecure attachment styles were more likely to make negative, internal attributions to the nature of the cry (e.g., spoiled or difficult temperament). In addition, they were less accurate at identifying infant emotions and more likely to be amused or neutral in response to infant distress. Other studies support the notion that insecure parents tend to process infant crying or other attachment-related information in a defensive and negatively biased manner, and that this kind of information processing contributes to insensitive interactions that increase the risk of insecure infant attachments (Dykas & Cassidy, 2011).

One way to operationalize behavioral responses to infant cry signals is using a hand-grip dynamometer to measure whether participants squeeze with excessive force when they are exposed to infant signals. For example, Crouch, Skowronski, Milner, and Harris (2008) showed that parents at risk for child abuse who were primed with hostile words tended to use more excessive force when they were exposed to videotaped segments of crying, smiling and quiet infants. Furthermore, the hand-grip dynamometer was used to study the effect of perceived control on punitive force when teaching a computer game to children believed to be at a distant location (Bugental, Lewis, Lin, Lyon, & Kopeikin, 1999). Women with low perceived control used high levels of punitive force when children were ambiguously responsive to their instructions. Excessive use of punitive force was interpreted as an analogue of reactive force to children's disobedience, which is in line with other studies that showed that high levels of handgrip force are related to socially dominant behaviors (Gallup, O'Brien, White, & Wilson, 2010). According to Bugental et al. (1999), adults who have a negatively distorted view of the motives of children might use exaggerated punitive force as a defense against the power of children. In a recent study, Bakermans-Kranenburg et al. (2012) found that oxytocin, a neuropeptide that is important for mother-infant bonding (Carter, 1998; Galbally, Lewis, Van IJzendoorn, & Permezel, 2011), reduced the use of excessive force during exposure to infant crying in individuals who experienced little harsh discipline in their childhood. Low levels of oxytocin have been observed in mothers with insecure attachment representations as assessed with the AAI (but coded with the Crittenden system, Strathearn, Fonagy, Amico, & Montague, 2009) and low oxytocin levels have been related to reduced sensitive responsiveness to infant signals (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Weller, Zagoory-Sharon, & Levine, 2007). In the present study we used the hand-grip dynamometer to assess the use of excessive force during exposure to infant crying in individuals with secure *versus* insecure AAI classifications. As insecure individuals tend to process infant cues in a more negative manner (Leerkes & Siepak, 2006), we expect that they will use more excessive force during exposure to infant crying than individuals with a secure representation.

The neural mechanism underlying the perception of infant cry signals has been the focus of several functional Magnetic Resonance Imaging (fMRI) studies. These studies have shown that a highly interactive cognitive-affective neural network is involved in the perception of infant crying (Bos, Panksepp, Bluthé, & van Honk, 2012). The amygdala is an important functional hub within this network. It is activated during exposure to infant crying (Lorberbaum et al., 2002; Riem et al., 2011; Seifritz et al., 2003) and it is connected with other brain regions that are involved in the perception and evaluation of crying such as the orbitofrontal cortex (OFC) and the anterior cingulate cortex (ACC) (Riem et al., 2012). The amygdala is part of the limbic system and is involved in the detection of threat and the experience of fear and aversion (Davis & Whalen, 2001; Fusar-Poli et al., 2009; Gamer, Zurowski, & Buchel, 2010; Morris et al., 1998). Heightened amygdala activation is an indication of hyperemotionality and has been observed in depression and anxiety disorders (Rauch et al., 2000; Yang et al., 2010) and in intrusive mothers (Atzil, Hendler, & Feldman, 2011). Several fMRI studies also indirectly point to a role of the amygdala in the processing of attachment-related information. For example, Buchheim et al. (2006) found elevated amygdala activation in individuals with unresolved loss during the Adult Attachment Projective (AAP). Increased amygdala responses have also been observed in individuals with high self-reported anxious attachment during the observation of angry facial expression conveying negative feedback about task performance (Vrtička, Andersson, Grandjean, Sander, & Vuilleumier, 2008).

However, self-report questionnaires have been shown to overlap only marginally with the AAI (Roisman et al., 2007), and little research has been conducted on the neurobiological processes underlying the perception of infant signals and AAI representations as classified with the extensively validated standard Main, Goldwyn and Hesse (2003) coding system. Previous fMRI studies on the influence of adult attachment representation mainly focused on the perception of infant facial expressions. Strathearn et al. (2009) examined neural responses to own infant smiling and sad faces in first-time mothers and found that mothers with an insecure attachment representation (as assessed with the Crittenden coding system) showed less activation in dopaminergic reward centers such as the ventral striatum compared with secure mothers. In a recent study, Lenzi et al. (in press) found that individuals with a dismissing attachment representation showed increased activation in the limbic and mirror neuron system and greater deactivation in the OFC and ACC in response to infant facial expressions compared with individuals with a secure representation, possibly reflecting dismissing mothers' affective dysregulation and lack of emotional investment in attachment relationships. Furthermore, Galynker et al. (2012) examined the influence of depression and attachment insecurity (measured with the AAI) on neural responses to images of the participant's mother, friend or a stranger. They found that insecure attachment was associated with enhanced activation in brain regions related to affectively motivated behavior and memory. However, no effects of attachment security on amygdala activation were found, possibly because of the absence of negative affective stimuli.

In the present study we investigated amygdala responses to infant crying in individuals with secure and insecure attachment representations, using the 'gold standard' for adult attachment, the AAI (Hesse, 2008). Considering that the amygdala seems to be involved in both adult attachment and the perception of infant signals, we examined whether amygdala hyperactivity mediates the relation between insecure attachment representation and negative emotional and/or behavioral responses to infant crying. In 10-year-old children amygdala hyperactivity was found to mediate the association between adverse early attachment experiences (growing up in institutionalized care) and decreased eye-contact during dyadic interaction (Tottenham et al., 2011). This indicates that early adverse attachment experiences affect amygdala activity, possibly because of the vulnerability of the amygdala to environmental exposures in early life (Lupien, McEwen, Gunnar, & Heim, 2009; Sabatini et al., 2007), which in turn influences social behavior. Amygdala hyperactivity might thus be one of the mechanisms underlying the association between adult attachment representation and emotional and/or behavioral responses to infant crying.

To our knowledge, this is the first study to investigate the influence of adult attachment representation as assessed with the AAI on neural, emotional and behavioral responses to infant crying. We examined amygdala activation, feelings of irritation, and the use of excessive force during exposure to infant crying. We hypothesized that 1) insecure individuals experience more irritation during the perception of infant crying than individuals with a secure representation; 2) insecure individuals use more excessive force as indicated by grip strength using a hand-grip dynamometer during exposure to infant crying; 3) individuals with insecure attachment representations show heightened amygdala activation during exposure to infant crying compared to individuals with a secure representation; 4) the relation between attachment representation and emotional or behavioral responses to infant crying is mediated by amygdala activation.

Method

Participants

Participants were selected from a larger study investigating caregiving responses and physiological reactivity to infant crying (Out, Pieper, Bakermans-Kranenburg, & Van IJzendoorn, 2010). The original sample consisted of 50 male and 134 female adult twin pairs. A group of 43 right-handed women, 21 from MZ twin pairs and 22 from DZ twin pairs were selected to participate in an fMRI study investigating the influence of oxytocin administration on neural responses to infant crying. Data for the current study were acquired from the 21 participants (12 MZ, 9 DZ, no pairs) who were randomly assigned to the placebo condition. Data regarding oxytocin effects on neural responding to infant crying has been presented elsewhere (Riem et al., 2011). Participants were screened for hearing impairments, MRI contraindications, pregnancy, psychiatric or neurological disorders, alcohol and drug use and did not have children of their own. At the time of fMRI data acquisition the mean age of the participants was 29.05 years (*SD* =7.55, range 22-49). 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 to the lab for 2 waves of data collection. In the first session, the AAI was administered in a quiet room. In the second session, fMRI data acquisition was performed and emotional and behavioral responses to infant crying were measured. After explaining the fMRI procedure 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. Participants were instructed to attend to the sounds they would hear in the fMRI scanner. After fMRI scanning participants rated how much irritation they felt while listening to the crying sounds, and the handgrip-force task was administered.

Measures

Adult Attachment Interview. Ratings and classifications of adult attachment representations were derived from the Adult Attachment Interview (AAI; Main et al., 1985, 2008), which was conducted during a lab session. The AAI is considered to be the gold standard for assessing attachment representations (Hesse, 2008). The AAI is an hour-long, semi-structured interview which assesses an individual's current state of mind with respect to attachment. Participants are asked about their childhood attachment experiences with their parents and how they think they were affected by these experiences, as well as about the current relationship with their parents. It is the coherence of discourse rather than the content of the autobiographical account that determines their attachment classification (see Hesse, 2008, for a detailed description of the assessment). Coding of the AAI yields one of three main adult attachment classifications: Secure-Autonomous (F), Insecure-Dismissing (Ds), and Insecure-Preoccupied (E). Adults with the F classification tend to value attachment relationships, to describe their attachment experiences (whether positive or negative) coherently, and to consider them important in the development of their personality. Adults with the Ds classification tend to idealize their childhood experiences without being able to provide concrete illustrations, or tend to minimize the importance of attachment in their own lives. Adults with the E classification tend to emphasize the impact, often negative, of their attachment experiences. They are still very much involved and preoccupied with these experiences. An additional classification, unresolved (U), is assigned when an interview shows signs of unresolved trauma or loss.

Interviews were audio-recorded, transcribed verbatim, and scored according to the standard AAI classification system (Main et al., 2008). The interviews were anonymously assigned and coded blindly by 3 raters who were trained to be reliable to the coding standards of the Berkeley laboratory of Mary Main and Erik Hesse. Scores for coherence of mind and unresolved trauma were are assigned using a nine-point rating scale (Hesse, 2008). Mean score for coherence of mind in the current sample was 4.53 (SD = 2.08). Seven participants were classified as secure, four participants as dismissing, four participants as preoccupied

and six participants as unresolved. Subjects were reclassified as either secure (autonomous) or insecure (dismissing, preoccupied, unresolved), resulting in a group of 14 insecure participants and a group of 7 secure participants. In addition, participants were reclassified as unresolved or not unresolved, resulting in a group of 6 participants with an unresolved state of mind and 15 participants without an unresolved state of mind. For one participant it was not possible to assign a coherence of mind score because some AAI questions were missing due to problems with audio-recording. The missing value of this participant, who was classified as secure by two independent expert raters, was replaced by the mean coherence of mind score of individuals with a secure classification.

Emotional and behavioral responses to infant crying. After fMRI scanning the participants were asked to report whether they felt irritated while listening to the crying sounds on a 5-point Likert scale (1 = not irritated, 5 = irritated). In addition, an adult hand dynamometer was used as an indicator of the use of excessive force during listening to infant crying. The dynamometer (model TSD121C) weighed 315 g and was 185-mm long, 42-mm wide and 30-mm thick, with an isometric range from 0 to 100 kg. Squeeze intensities (in kg) were transferred directly from the dynamometer to the AcqKnowledge software program (version 3.8; Biopac Systems, 2004). Matlab (version 7.8.0, Mathworks, MA, USA) was used to identify peak intensities for each squeeze. Participants were asked to squeeze the handgrip dynamometer as hard as possible and then at 50% of their maximal handgrip strength. They performed as many trials as necessary for training, with their performance displayed on a monitor to check the 50% level of each second handgrip, until they were able to modulate the force of their second squeeze to half the strength of their first squeeze. Then the monitor was directed away from the participant in order to prevent them from receiving feedback regarding their performance during the remainder of the task.

The handgrip-force task was administered on a laptop using E-Prime software (version 2.0; Psychology Software Tools, Inc., PA, USA). During the task participants were seated in front of a computer screen wearing headphones (type König CMP). As a prompt, the words 'squeeze maximally' were displayed briefly in the middle of the screen, after 2 s followed by the prompt 'squeeze at half strength', thus prompting the participants to perform a brief firm squeeze followed by a brief squeeze half the strength. After baseline squeezing (no sound), participants were requested to squeeze the handgrip dynamometer eight times at full and half strength, respectively, the first four times listening to infant laughter and then four times listening to infant crying. In the current study we focus on squeezing during infant crying only. Squeezing differences during infant crying and laughter between the placebo and the experimental group are presented elsewhere (Bakermans-Kranenburg et al., 2012).In that report, attachment representations have not been presented because they were not yet available at that time. The infant laughter sound (duration = 2 min, average fundamental frequency = 215.96 Hz, constant volume) and the infant crying sound (duration = 2 min, average fundamental frequency = 360.06, constant volume) from Groh and Roisman (2009) were used. The intervening time between full- and half-strength

prompts was 2 s; the intervening time period between half-strength and the next full-strength prompt was 25 s. Similar to Bakermans-Kranenburg et al. (2012) and Crouch et al. (2008), grip strength modulation was calculated by dividing the half-strength squeeze intensity by the full-strength squeeze intensity, so that scores of over 0.50 indicated excessive force on the half-strength squeeze attempt. As a result of fatigue the last trial yielded too many missing data. Therefore we decided to use the first three trials during infant crying, for which we added the numbers of trials with too much physical force (>0.50).

Neural responses to infant crying. Blood oxygenation-level dependent responses to infant crying were measured with fMRI. Participants were instructed to attend to the sounds they would hear and they listened to the sounds through MRI compatible headphones. Cry sounds were derived from the spontaneous crying of a healthy 2-day old infant. A 10-s 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-s 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 (Dessureau, Kurowski, & Thompson, 1998; Out et al., 2010; Schuetze & Zeskind, 2001; Schuetze, Zeskind, & Das Eiden, 2003). 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 (Soltis, 2004). We did not expect to find differences in brain activation between the frequency conditions, as there was no significant effect of frequency in a previous study on neural responses to infant crying (Riem et al., 2011). 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. The participants did not perceive much irritation during exposure to the control sounds collapsed across frequencies (M = 1.95, SD = 1.27) and there was no significant difference in reported irritation between secure and insecure individuals ($t(19)$ = -0.72, p = .48). 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 s. Cry sounds were collapsed across pitches to reduce the number of statistical tests.

FMRI data acquisition and analysis

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. Cushions were placed between the head coil and the participant in order to prevent head movement. First, a T1-weighted anatomical scan was acquired (flip angle = 8°, 140 slices, voxelsize .875×.875×1.2 mm). For fMRI, a total of 360 T2*-weighted whole-brain EPIs were acquired (TR = 2.2 s; TE = 30 ms, flip angle = 80°, 38 transverse slices, voxelsize 2.75×2.75×2.75 mm (+10% interslice gap)). 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.

Data analysis was carried out using FEAT (FMRI Expert Analysis Tool) Version 5.98, part of FSL (FMRIB's Software Library, www.FMRIb.ox.ac.uk/fsl; (Smith et al., 2004). The following pre-statistics processing was applied: motion correction (Jenkinson, Bannister, Brady, & Smith, 2002), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 5.0 mm, and high-pass temporal filtering (highpass filter cutoff = 50.0 s). Functional scans were registered to T1-weighted images, which were registered to standard space (Jenkinson et al., 2002; Jenkinson & Smith, 2001).

In native space, functional activation was examined using 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 gamma hemodynamic response function and its temporal derivative was added to the model, giving 12 predictors. The contrast $\text{Cry}_{\text{combined 500, 700, 900 Hz}} > \text{Control}_{\text{combined 500, 700, 900 Hz}}$ was assessed. Data regarding functional activation of other brain regions during exposure to infant crying at different frequencies is presented elsewhere (Riem et al., 2011). The first-level contrast images and the corresponding variance images were transformed to standard space and submitted to second-level mixed-effects group region of interest analyses with the right and left amygdala (>50% probability, defined using the Harvard–Oxford subcortical atlas, http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). Centered coherence of mind scores were added to the model as a regressor and we assessed the positive and the negative contrast of this regressor to examine the positive and negative correlation between functional activation during infant crying (versus control sounds) and coherence of mind. Furthermore, in an additional analysis we tested for group differences using two-sample t-tests on the Cry > Control contrast with the unresolved versus not unresolved comparison (unresolved > not unresolved and unresolved < not unresolved). Age and menstrual cycle (centered) were included 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 $p < .05$ (Worsley, 2001). Mean Z values for voxels that were significantly related to coherence of mind during infant crying compared with control sounds were derived using Featquery (FMRIb.ox.ac.uk/fsl/feat5/featquery.html). Thus, the mean Z value was calculated across the significant cluster falling within the amygdala region of interest.

Results

Emotional and behavioral data

Irritation. In Table 1 the means of emotional and behavioral responses to infant crying for each attachment classification are presented. Coherence of mind tended to be related to reported irritation during exposure to infant crying (r = -.37, p = .10),. Similarly, insecurely attached individuals tended to report more irritation during exposure to infant crying compared with securely attached individuals ($t(19) = 1.73$, $p = .10$) (insecure: $M = 3.83$, $SD = 1.21$, secure: $M = 2.86$, $SD = 1.24$;). Effect sizes for the association between feelings of irritation and coherence or

security of attachment were large (Cohen's *d* amounted to 0.80 in both cases). The four-way attachment classification (F, Ds, E, U) did not have a significant effect on reported irritation during the exposure to infant crying ($F(3,17) = 1.87$, $p = .17$). Neither was there a significant difference in reported irritation between individuals with an unresolved state of mind versus without an unresolved state of mind during exposure to crying ($t(19) = 0.99$, $p = .34$, d = 0.48).

Handgrip force. We examined whether attachment representation was related to excessive use of handgrip force during exposure to infant crying. We found a significant negative correlation between coherence of mind and excessive use of handgrip force ($r = -.47$, $p < .05$). In addition, there was a significant difference between securely and insecurely attached individuals on excessive use of handgrip force ($t(19) = 3.11$, $p < .01$, Cohen's $d = 1.38$). Insecurely attached individuals more often used excessive handgrip force ($M = 2.57$, $SD = 0.65$) compared with securely attached individuals ($M = 1.57$, $SD = 0.79$). Furthermore, the four-way attachment classification showed significant differences in excessive handgrip force ($F(3,17) = 4.06$, $p < .05$). A priori contrasts showed that individuals with a preoccupied attachment representation more often used excessive force during exposure to infant crying compared with individuals with a secure attachment representation ($t(6.00) = 4.80$, $p < .01$, unequal variances). In addition, the difference between secure individuals and individuals with a dismissing attachment representation ($M = 2.50$, $SD = 0.58$) was marginally significant ($t(8.15) = 2.24$, $p = .06$, unequal variances). The difference between individuals with an unresolved state of mind versus individuals with a secure attachment representation for use of excessive handgrip force was not significant ($t(10.56) = 1.71$, $p = .12$, unequal variances). There was no significant difference in use of excessive handgrip force between individuals with an unresolved state of mind versus without an unresolved state of mind ($t(19) = 0.33$, $p = .75$).

In addition, to test whether the effects of attachment representation on handgrip force were specific to infant crying, we also examined use of excessive force during exposure to infant laughter. The interaction between group (secure, insecure) and condition (crying, laughter) was significant, $F(1,19) = 7.03$, $p = .016$. During the laughter condition there was no significant difference in use of excessive force between secure and insecure individuals ($t(19) = -0.62$, $p = .54$).

Table 1. Means (*M*) and standard deviations (*SD*) of reported irritation and handgrip force in response to cry sounds for individuals with a secure (F), dismissing (Ds), preoccupied (E) and unresolved (U) attachment representation.

Attachment classification	*n*	Irritation to Cry		Handgrip force	
		M	*SD*	*M*	*SD*
F	7	2.86	1.24	1.57	0.79
Ds	4	4.42†	1.17	2.50†	0.58
E	4	3.08	1.62	3.00**	0.00
U	6	3.94†	0.83	2.33	0.82

Higher than F: $^{\dagger}p < .10$, **$p < .01$

Neuroimaging data

The contrast of infant crying (500 Hz, 700 Hz, 900 Hz) versus control sounds (500 Hz, 700 Hz, 900 Hz) revealed significant activation in the region of interest analysis of the right amygdala (Cluster size = 66 voxels, peak Z = 3.22, MNI coordinates x,y,z (mm) = 22, -8, -16) ($p < .05$, corrected by cluster threshold ($Z > 2.3$). In addition, we found a significant negative correlation ($r = -.56$, $p < .01$) between coherence of mind and right amygdala activation during exposure to infant crying compared to control sounds (Cluster size = 18 voxels, peak Z = 2.77, MNI coordinates x,y,z (mm) = 20, -2, -22) (see Figure 1). Higher scores for coherence were related to less right amygdala activation. The left amygdala was not significantly activated during infant crying compared to control sounds and there was no significant correlation between left amygdala activation and coherence of mind. Furthermore, there was no significant difference in amygdala activation between individuals with an unresolved state of mind versus individuals without an unresolved state of mind.

An ANOVA was performed to examine the effect of adult attachment representation on mean Z-values extracted from the right amygdala. Adult attachment representation was significantly associated with amygdala activation ($F(3,17) = 3.31$, $p < .05$). A priori contrasts showed that individuals with preoccupied and dismissing attachment representations showed increased amygdala activation during infant crying (relative to control sound) compared with individuals with a secure attachment representation (E: $t(17) = 2.50$, $p < .05$, Cohen's $d = 2.35$, Ds: $t(17) = 2.67$, $p < .05$, Cohen's $d = 2.83$). However, individuals with an unresolved state of mind did not show increased amygdala activation compared with individuals with a secure attachment representation ($t(17) = 1.31$, $p = .21$) (see Figure 2) and there was no significant correlation between amygdala activation and scores on unresolved loss ($r = -.03$, $p = .90$).

We examined the correlation between amygdala activation and reported irritation and use of excessive force in order to investigate whether amygdala activation mediated the relation between attachment representation and emotional or behavioral responses to infant crying. There was no significant correlation between amygdala activation and reported irritation during exposure to infant crying or between amygdala activation and use of excessive force, thus indicating that associations between attachment representation and reported irritation or handgrip force was not mediated by amygdala activation.

Discussion

Our study is the first to validate the standard Berkeley Adult Attachment Interview with functional Magnetic Resonance Imaging by demonstrating that adult attachment representation influences neural, emotional and behavioral responses to infant crying. Individuals with an insecure attachment representation showed heightened amygdala activation during exposure to infant crying compared with individuals with a secure attachment representation. In addition, insecure individuals tended to experience more irritation during the perception of infant crying and they used more excessive force as indicated by grip strength

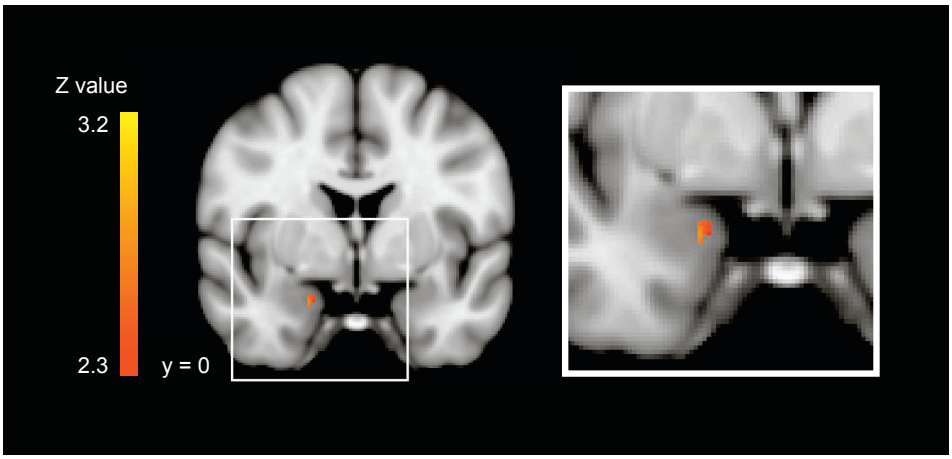

Figure 1. Significant correlation between coherence of mind and right amygdala activation during exposure to infant crying (500, 700, 900 Hz) compared with control sounds (500, 700, 900 Hz). Region of interest analysis, $p < .05$, corrected by cluster threshold ($Z > 2.3$). The right side of the brain corresponds to the left hemisphere and vice versa.

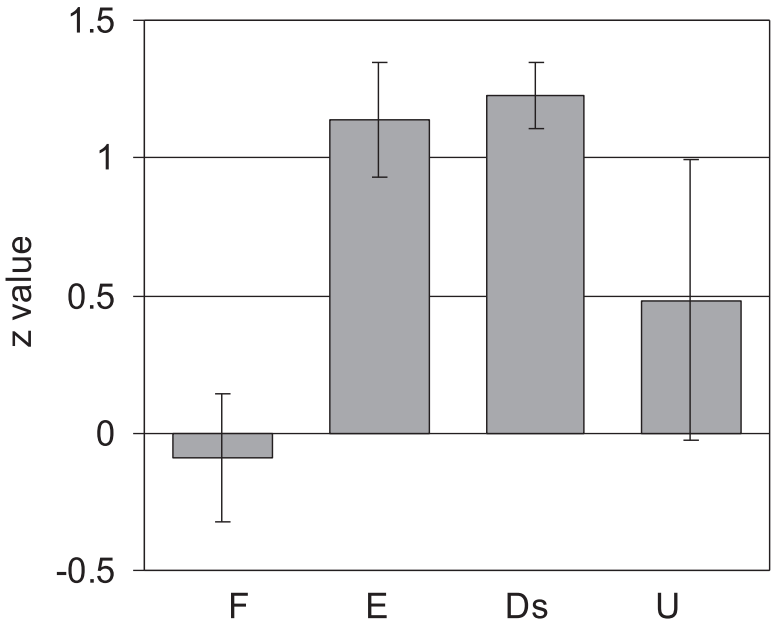

Figure 2. *Z*-values (*M, SE*) of right amygdala activation during infant crying compared with control sounds for individuals with a secure ($n = 7$), preoccupied ($n = 4$), dismissing ($n = 4$) and unresolved state of mind ($n = 6$).

using a hand-grip dynamometer compared with individuals with a secure representation. Amygdala hyperactivity might explain why insecure individuals experience more aversive and angry feelings during exposure to infant crying and why they respond inconsistently to infant signals or reject their infants' attachment behavior. Our findings indicate that differences between attachment classifications can be observed at the neural level and extend previous studies that used physiological measures such as skin conductance and autonomic reactivity to validate the AAI (Beijersbergen, Bakermans-Kranenburg, Van IJzendoorn, & Juffer, 2008; Dozier & Kobak, 1992).

Infant crying has been described as a paradoxical signal (Soltis, 2004). It elicits warm, empathic feelings in parents and enhances infant survival by stimulating parental proximity and care (Bowlby, 1969/1982; Dix, 1991). Maternal sensitivity to infant crying has been shown to predict infant attachment security with more explanatory power than maternal sensitivity to infant signals in non-distress settings (McElwain & Booth-LaForce, 2006), suggesting that infant crying plays a crucial role in the formation of the attachment bond between mother and child.

On the other hand, crying also elicits negative emotions such as aversion and anger (Dix, 1991; Dix et al., 2004) and excessive infant crying can even trigger child abuse and neglect (Soltis, 2004). In the Netherlands, six months after the infant's birth nearly 6 % of the parents report that they have shaken, smothered or slapped their infant in order to stop the crying (Reijneveld et al., 2004). The likelihood of using insensitive parenting responses is increased when parents have strong negative emotional reactions to crying (Dix et al., 2004). These negative emotions undermine sensitive child-oriented parental responses such as feeding or soothing the child. Thus, parental negative emotional reactions to crying may play a crucial role in the development of insecure infant attachment. Our findings that insecure individuals tend to experience more irritation and use more excessive force during exposure to infant crying support this notion and are in line with previous research showing that insecure individuals are less accurate at identifying infant emotions and more likely to make negative attributions about a crying infant.

In addition, our findings also converge with event-related potential (ERP) studies on neural responding to infant signals. For example, Fraedich, Lakatos and Spangler (2010) examined event-related potentials during the presentation of infant emotion faces in mothers with secure and insecure attachment representations, measured with the Adult Attachment Projective (AAP) (George, West, & Pettem, 1999). They found that insecure mothers showed a more pronounced negativity in the face sensitive N170 component, and smaller N200 and P300 amplitudes in response to infant faces. This might indicate that secure mothers have a more efficient face perception and allocate more attention to social and face stimuli. However, in terms of format, structure and content the AAP is not isomorphic to the AAI, and the load of validating evidence for the latter still has to emerge for the AAP. In a study using ERPs in response to neutral, happy and fearful faces Zhang Li, and Zhou (2008) found that avoidant Chinese undergraduate students showed different N1, N2, P2, and N400 components compared to secure or anxious students, suggesting differences in both earlier,

automatic encoding of faces and later, more elaborative retrieval of emotional information. However, they used the Experiences in Close Relationships scale to assess attachment style.

Few studies have been conducted on adult attachment representations associated with neural responding to infant signals. Only one study investigated the neurobiological mechanism underlying the perception of infant stimuli in individuals classified with the standard Main, Goldwyn and Hesse (2003) coding system. In this study, Lenzi et al. (in press) examined neural activation during observing and imitating infant facial expression in individuals with secure and dismissing attachment representations. Individuals with dismissing attachment representations showed more activation in motor, limbic and mirror brain regions, indicating that they were more emotionally reactive to infant stimuli than secure individuals. In contrast to the present study, insecure individuals did not show increased amygdala activation, possibly because individuals were presented with happy, neutral and distressed infant faces. Infant crying is one of the most important attachment behaviors, alerting parents when the infant is in danger (Soltis, 2004). Therefore, it might be more emotionally salient than visual infant stimuli, with larger individual differences in amygdala activation as a result.

In a study using the Crittenden coding system for adult attachment, dismissing mothers showed increased activation in brain regions related to disgust and decreased activation in neural reward areas in response to happy and sad infant faces. Unfortunately, the use of the Crittenden coding system hampers the comparability with the findings in the present study, and with a host of other validating evidence for the AAI. Although our results are in line with two other neuroimaging studies that point to a role of the amygdala in insecure attachment, these studies used the AAP or self-report measures of attachment (Buchheim et al., 2006; Vrtička et al., 2008). Self-report measures and the Adult Attachment Interview may not be used interchangeably to examine the neural base of attachment because they have little empirical or conceptual overlap (Roisman et al., 2007). Greater convergence in the way in which adult attachment is measured and what paradigm is used in the fMRI sessions would advance our understanding of the mechanisms underlying attachment representations and parenting.

Contrary to our expectations, the relation between attachment representation and emotional or behavioral responses to infant crying was not mediated by amygdala activation. This seems to indicate that feelings of irritation and the use of excessive force in response to infant crying in insecure individuals can not be solely explained by a hyperactive amygdala. Other brain regions might be involved in attachment-related influences on the perception of infant crying, for example brain regions important for empathy and emotion understanding such as the insula and the inferior frontal gyrus (IFG). In a previous study, we found that intranasal administration of oxytocin, a hormone that enhances parental sensitivity and parent-infant bonding (Naber, Van IJzendoorn, Deschamps, Van Engeland, & Bakermans-Kranenburg, 2010), decreased amygdala responses and increased insula and IFG responses to infant crying (Riem et al., 2011). These

findings point to a role of empathy-related brain regions in sensitive parenting that might be associated with adult attachment representations.

Alternatively, since our control sounds were neutral and lacked human-like connotations, it is hard to know exactly what aspect of infant crying is being reflected in the neural responses, as the cry sounds may reflect a composite of factors (human sounds, infant sounds, distressing sounds). The amygdala response might thus be relatively non-specific whereas the emotional and behavioral responses may have been more specific to the distressing components of infant crying. In that case it would not be surprising that the amygdala responses did not mediate the relation between attachment representation and responses to crying. Indeed, it should be noted that the emotional and behavioral responses were observed in reaction to the cry sounds in comparison to infant laughter. To test the alternative interpretation emotional and behavioral responses should be registered in a comparison between infant cry and scrambled sounds. It should be noted, however, that there is a limit to the number of stimuli that can be presented within and outside the scanner.

Another explanation for the finding that the amygdala did not mediate the relation between insecure adult attachment and emotional and behavioral responses to crying might be that disruptions in amygdala *connectivity* also play a role in the negative perception of infant crying in insecure individuals. The amygdala is strongly connected with other brain regions within a neural network involved in the perception and evaluation of crying (Riem et al., 2012), and neural disorganization within this network has been associated with anxious parenting (Atzil et al., 2011). Tottenham et al. (2011) found that amygdala hyperactivity to fearful faces mediated the relation between adverse rearing experiences and decreased eye-contact during dyadic interaction, and they suggested that early adversity may have affected amygdala development and caused long-term structural abnormalities (Sabatini et al., 2007). Adult attachment and early rearing experiences have been shown to be distinct constructs since experiences in subsequent social relationships influence how individuals represent past and present attachment experiences (Waters, Hamilton, & Weinfield, 2000; Weinfield, Sroufe, & Egeland, 2000). Therefore, systematic differences in structural amygdala development are unlikely to be found in individuals with different attachment classifications in non-clinical samples.

Our finding that the amygdala plays a role in the perception of crying in individuals with insecure attachment representation is not consistent with the proposition that the attachment system is located in the orbitofrontal cortex (OFC) (Schore, 2001). The OFC is involved in reward processing, emotional regulation, and the perception of infant signals (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Kringelbach, 2005; Kringelbach et al., 2008; Stein et al., 2007). For example, it exhibits a very rapid and specific response to an infant face and it has been suggested that this might be the brain basis for the "innate releasing mechanism" described by Lorenz (Kringelbach et al., 2008). However, it is by no means the only brain region involved in attachment. By using Wittgensteins analogy, Coan (2008) suggested that searching for the identification of a single attachment neural construct is like 'trying to find the real artichoke by peeling

away all its leaves'. Because so many brain regions are involved in attachment, it can not be reduced to a single neural construct. For example, parental responses to infant crying require activation of multiple cortical and subcortical neural systems involved in functions ranging from the processing of visual and auditory information to complex processes such as affection, emotional regulation, and memory. Moreover, efficient interaction between these systems is needed in order to respond to a crying infant in a sensitive way (Atzil et al., 2011; Riem et al., 2012). Thus, the attachment system most likely relies on a comprehensive neural network and insensitive responsiveness in insecure adults can not be explained by malfunctioning or dysregulation of a single neural construct.

The limitations of this study should be noted. First, our findings can only be generalized to women without children. The choice for women without children increased the comparability of our participants in terms of their experiences with infant crying. Attachment-related influences on emotional, behavioral and neural responding to infant crying might be even more pronounced when parents are exposed to their own infant's crying. For example, Seifritz et al. (2003) showed that neural responses to infant vocalizations dramatically changed with parental status, with parents showing more amygdala activation than nonparents. Second, as we compared neural responses to infant crying with neutral control sounds, it is unclear whether insecure individuals show amygdala hyperactivity specifically during exposure to infant crying, or whether the amygdala is also hyperactive during other vocal emotional stimuli. However, our finding that insecure individuals do not show excessive force in response to infant laughter indicates that the effects of attachment representations may be more pronounced during exposure to infant crying compared with other emotional stimuli. Another limitation of the present study is the small sample size, which led to the combination of insecure classifications in the analyses and to relatively low power for the analyses. The large effect sizes for the association between coherence or security of attachment and feelings of irritation would have reached significance in a somewhat larger sample. Since the combination of time-consuming AAI research and an expensive fMRI investigation leads to an almost impossible mission for a single research group we hope that we will be joined in the next future by other teams, examining the neurobiological processes underlying the separate dismissing, preoccupied and unresolved attachment representations in larger samples. Furthermore, future studies may examine the role of other brain regions and the functional connectivity between brain regions in individuals with different attachment representations. The amygdala has often been described as a functional hub because of its high degree of connectivity with other brain regions. In a previous study, we found that the oxytocin enhances connectivity between the amygdala and the OFC and the ACC during exposure to infant laughter, indicating that efficient amygdala connectivity might be one of the mechanisms underlying sensitive responsiveness (Riem et al., 2012). Disruptions in amygdala connectivity have been observed in patients with depression and anxiety disorders (Dannlowski et al., 2009; Pillay, Gruber, Rogowska, Simpson, & Yurgelun-Todd, 2006) and might also play a role in insecure attachment representations.

In conclusion, our study is the first to show that neural differences in response to infant crying are associated with adult attachment representations. Our results provide fMRI validation of the Adult Attachment Interview and extend previous behavioral validation studies. We found that insecure individuals tended to experience more irritation, and they used more excessive force as indicated by grip strength and showed heightened amygdala activation during exposure to infant crying compared to individuals with a secure representation. Amygdala hyperactivity might be one of the mechanisms underlying the experience of negative emotions during exposure to infant crying in insecure individuals and might explain why insecure parents have more difficulty responding to their crying infant in a sensitive way.

5 Pity or Peanuts? Oxytocin affects neural response to sick and bored infant crying

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Abstract

The neuropeptide oxytocin plays an important role in mother-infant bonding. However, recent studies indicate that the effects of oxytocin on prosociality are dependent on perceived social context. Using functional magnetic resonance imaging, we examined differential effects of intranasally administered oxytocin on neural responding to 500 and 700 Hz crying that was indicated as emanating from a sick infant and 500 and 700 Hz crying emanating from a bored infant. We found that oxytocin significantly increased insula and inferior frontal gyrus responding to sick infant crying, but decreased activation in these brain regions during exposure to crying of an infant that was labeled as bored. In addition, oxytocin decreased amygdala responding to 500 Hz crying, but increased amygdala responding to 700 Hz crying. These findings indicate that oxytocin enhances the salience of the context and of the acoustics of crying, thus facilitating the interpretation of the cry and the selection of an adequate caregiving response.

Introduction

Infant crying is evolutionary adaptive because it elicits parental proximity and care, and because it conveys information about the health condition of the child (Soltis, 2004). Specific neural circuits may have evolved in caregivers to facilitate perception and evaluation of infant crying (Seifritz et al., 2003). In a previous study, we found that the neuropeptide oxytocin sensitizes caregivers to variations in cry signals by modulating these neural circuits and enhancing sensitive responsiveness to crying (Riem et al., 2011). Other studies have shown that intranasally administered oxytocin stimulates a range of social behaviors, including trust, empathy, and emotion understanding (for a review see De Dreu, 2012). However, recent studies also indicate that the effects of oxytocin on prosociality are dependent on social context (De Dreu, 2012) and personal history (Van IJzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, & Tops, 2011), possibly because oxytocin increases salience of social information (Graustella & MacLeod, 2012). Using functional magnetic resonance imaging (fMRI), we examined differential effects of oxytocin on neural responding to infant crying with varying information on the context of crying.

Infant crying has been described as a graded signal that changes as a function of the level of distress of the infant (Gustafson, Wood, & Green, 2000). For example, infants who are in pain cry at higher fundamental frequencies than infants who are hungry (Soltis, 2004). In addition to the acoustics of the cry, a range of other factors may influence parental responses to infant crying, such as infant facial expressions, gestures, and contextual information. Indeed, maternal responses to crying have been shown to be delayed when the infant has just been fed, indicating that knowledge of the infant's recent caregiving history influences behavioral responses to crying (Bernal, 1972; Leger, Thompson, Merritt, & Benz, 1996). Caregiving context has also been shown to influence caregiving responses to crying in an experimental setting: Adults who had been told that an infant needed sleep waited longer to respond to infant crying than those without this information (Wood and Gustafson, 2001). Thus, parents are not only sensitive to acoustic variations in crying but also to contextual information, and both sources of information are used to select a caregiving response (Soltis, 2004).

An important role in sensitive parenting is attributed to the neuropeptide oxytocin. Feldman et al. (2007) showed that maternal oxytocin levels across pregnancy are predictive of higher quality of postpartum maternal behavior. Oxytocin has shown anxiolytic and stress-reducing effects in breastfeeding mothers (Heinrichs et al., 2001) and this might promote parents' sensitivity to infant crying. Indeed, in a previous study we found that intranasal oxytocin may facilitate sensitive responding to crying by decreasing activation of the amygdala (Riem et al., 2011), a brain region involved in the experience of anxiety and aversion (LeDoux, 2000). Oxytocin also enhanced activation in the insula and inferior frontal gyrus (IFG), brain regions important for empathy and emotion understanding (Lamm, Decety, & Singer, 2011).

However, oxytocin might not enhance social behavior similarly for everyone and under all circumstances. For example, we found that intranasal oxytocin

decreased the use of excessive handgrip force in response to infant crying, but only in individuals with supportive family backgrounds, indicating that prosocial effects of oxytocin were moderated by harsh caregiving experiences (Bakermans-Kranenburg, Van IJzendoorn, Riem, Tops, & Alink, 2012). Other studies indicate that the social context may be crucial in shaping the effects of oxytocin on social cognition (Bartz, Zaki, Bolger, & Ochsner, 2011). For example, the trust-enhancing effects of oxytocin disappear when partners are unknown (Declerck, Boone, & Kiyonari, 2010). Some studies even point to negative effects of oxytocin on prosocial behavior. De Dreu et al. (2010) found that oxytocin enhanced in-group trust but also promoted defensive aggression toward individuals perceived as out-group members. Thus oxytocin might drive a "tend and defend" response (Carter, 1998), especially when out-group threat is high (De Dreu, Greer, Handgraaf, Shalvi, & Van Kleef, 2012).

One mechanism that might underlie these contextual and personal history influences on oxytocin effects is increased salience of social information (Graustella & MacLeod, 2012). Several studies indicate that oxytocin improves processing of social information and increases attention towards social cues (e.g., Rimmele, Hediger, Heinrichs, & Klaver, 2009). Increased salience of social information might explain the differential effects of oxytocin on behavior in different social contexts. Social context has a stronger influence on behavior when one is more aware of the contextual social cues. More specifically related to infant crying, oxytocin might increase the salience of the context of crying, thereby affecting the perception of and responding to the infant cry. Increased attention towards the context of the cry would be highly adaptive, as it facilitates the interpretation of the infant's crying and helps in selecting an adequate caregiving response. Thus, oxytocin may promote mother-infant bonding by increasing vigilance to contextual cues, which in turn leads to caregiving responses that are fine-tuned to the context of infant crying.

To our knowledge, this is the first study to investigate the influence of intranasally administered oxytocin on the perception of infant crying in systematically varied contexts. We examine differential effects of oxytocin on neural responding to crying that was indicated as coming from a sick infant and crying coming from a bored infant. We focus on neural responses to infant crying at different frequencies, because infant cries range from 500 Hz in normal, healthy infants to 700 Hz and even higher in infants in pain or with medical conditions. We were specifically interested in amygdala, insula and IFG responses to crying, since these regions were affected by intranasal oxytocin during exposure to crying in a previous sample (Riem et al., 2011). We expected to find enhanced neural responding to crying of a sick infant compared to crying of a bored infant, as crying related to sickness may be more alarming to parents, and more important from an evolutionary perspective (Soltis, 2004). Since oxytocin has been shown to enhance attention towards social information, we predicted that intranasal oxytocin would lead to increased processing of the contextual cues, leading to more pronounced differences in neural responding to sick infant crying compared to bored infant crying.

Method

Participants
A total of 343 female undergraduate students from the departments of education and child studies, and psychology at Leiden University participated in the first phase of the study. In this phase, the participants completed online questionnaires on their perception of parenting by their mothers, and some demographic details. One participant was excluded due to random responses. Five females with children of their own were also excluded. One hundred eighty six students participated in the second phase of the study, which was designed to examine behavioral and cardiac responses to infant crying. Fifty-four participants with scores ranging from low to high on a parenting questionnaire were selected to participate in the third phase of the study, consisting of a computer game designed to study prosocial helping behavior towards an excluded adult and for the current fMRI study. Participants were screened for MRI contraindications, psychiatric or neurological disorders, hearing problems, pregnancy, and alcohol and drug abuse. Four participants were excluded from the fMRI study because of hearing problems, resulting in a total sample size of 50 participants for the current study. They were randomly assigned to the oxytocin or the placebo condition. (n = 26 oxytocin, n = 24 placebo). The mean age of the participants was 19.66 years (SD =1.47, range 18-27). The majority (70 %) of the participants used oral contraceptives. Permission for this study was obtained from the Ethics Committees of the Institute of Education and Child Studies of Leiden University and of the Leiden University Medical Centre.

Procedure
Participants were invited for the fMRI study preferably in the luteal phase of their menstrual cycle in order to control for influences of menstrual cycle. For two participants it was not possible to determine menstrual phase, because of use of Mirena intrauterine device.

Approximately 35 minutes before the start of fMRI data acquisition participants took 6 puffs of nasal spray containing oxytocin (16 IU total) or 6 puffs of a placebo spray under supervision of the experimenter. Effects of 16 IU of oxytocin on social behavior and neural activity have been reported in previous studies (Riem et al., 2011; Van IJzendoorn, Bhandari, Van der Veen, Grewen, & Bakermans-Kranenburg, in press). Drug administration was double-blind. After nasal spray administration, the participants were familiarized with the task during practice trials outside the MRI scanner. Before drug administration and after fMRI scanning participants completed a mood questionnaire in order to track mood changes (see Supplementary Material).

Cry paradigm
Participants listened to cry and control sounds at 500 and 700 Hz (see Supplementary Material). Effects of oxytocin on neural responding to these crying sounds (and crying at 900 Hz) were found in a previous study (Riem et al., 2011). A bright green star was presented for 1 s in order to attract participants' attention

to the center of the screen (see Figure 1). Information about the context of the sound that would follow was presented on the computer screen for maximum 2 s. Context information consisted of information about the reason why the infant was crying: sickness ("This infant is sick") or boredom ("This infant is bored"). Context information was also presented for the control sounds at 500 and 700 Hz and consisted of neutral information about the sound: "This is a saw". In order to ensure that participants were attentive to the context information, they were instructed to press different buttons on a MRI compatible button box during the presentation of the three context conditions (sick, bored, saw). The task was self-paced. That is, the context information was followed by a cry or control sound when one of the buttons had been pressed. The crying sounds at 500 Hz and 700 Hz were presented in the sick as well as the bored crying condition. Cry and control sounds were presented in eight cycles, each cycle consisting of 6 trials. The order of presentation of conditions and sounds within each cycle was random; the intertrial interval was 4.5 to 7.5 s (M = 6 s, randomly jittered). A fixation cross was presented on the screen during intertrial intervals and sound presentation.

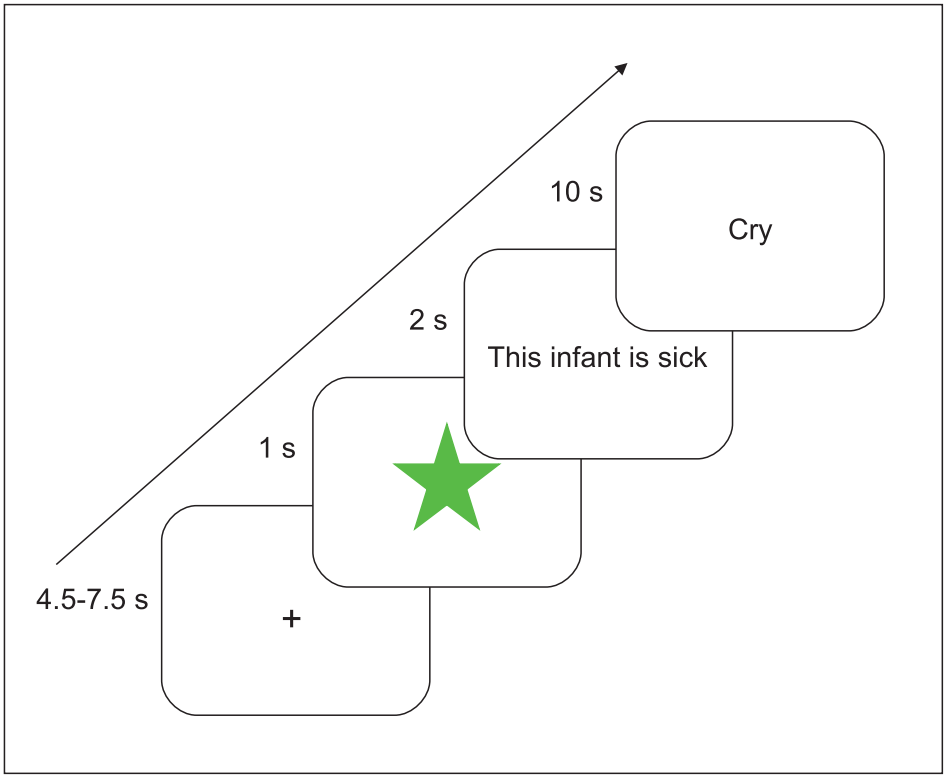

Figure 1. The cry paradigm. Participants' attention was attracted to the center of the screen by presenting a green star, followed by the context information. Context information ("This infant is sick", "This infant is bored", "This is a saw") was presented for maximum 2 s and was followed by the cry or control sounds after a button had been pressed. A fixation cross was presented on the screen during intertrial intervals and sound presentation.

fMRI data acquisition

Scanning was performed with a standard whole-head coil on a 3-T Philips Achieva TX 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 x .875 x 1.2 mm). For fMRI, a total of 422 T2*-weighted whole-brain echoplanar images were acquired (repetition time = 2.2 s; echo time = 30 ms, flip angle = 80°, 38 transverse slices, voxel size 2.75 x 2.75 x 2.75mm (+ 10% interslice gap)). Participants listened to the sounds through MRI-compatible headphones.

fMRI data analysis

Data analysis was carried out using FEAT (FMRI Expert Analysis Tool) version 5.98, part of FSL (Smith et al., 2004). The following pre-statistics processing was applied: motion correction (Jenkinson, Bannister, Brady, & Smith, 2002), non-brain removal (Smith, 2002), spatial smoothing using a Gaussian kernel of full-width-at-half-maximum 5.0 mm, and high-pass temporal filtering (highpass filter cutoff = 90.0s). Functional scans were registered to the high-resolution EPI-images, which were registered to the T1-weighted images, which were registered to standard space (Jenkinson et al., 2002).

In native space, functional activation was examined using general linear model analysis. Each sound condition (sick infant crying 500 Hz, sick infant crying 700 Hz, bored infant crying 500 Hz, bored infant crying 700 Hz, control sound 500 Hz, control sound 700 Hz) was modeled separately as a square-wave function. The star and the context information were also modeled as square-wave functions. Each predictor was then convolved with a double gamma hemodynamic response function and its temporal derivative was added to the model, giving 16 regressors. To examine regions involved in the perception of infant crying in context we assessed six contrasts: 1) sick infant crying 500Hz > control sound 500Hz, 2) sick infant crying 700Hz > control sound 700Hz, 3) bored infant crying 500Hz > control sound 500Hz, and 4) bored infant crying 700Hz > control sound 700Hz, 5) sick infant crying 500Hz > bored infant crying 500Hz, 6) sick infant crying 700Hz > bored infant crying 700Hz.

All 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 we tested for group differences using two-sample t-tests on these contrasts with the oxytocin versus placebo group comparison (Oxytocin > Placebo and Oxytocin < Placebo). We included menstrual cycle (follicular or luteal phase) and use of oral contraceptives as confound regressors in the model in the analyses of the group means and group differences. 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 *a priori* specified regions of interest were calculated for the first four contrasts using Featquery. These regions were the left insula, left inferior frontal gyrus pars opercularis and the right amygdala (Bos, Panksepp, Bluthé, & Honk, 2012; Riem et al., 2011), anatomically defined using the Harvard–

Oxford (sub)cortical atlas (http://www.fmrib.ox.ac.uk/fsl/data/atlas-descriptions.html#ho). In order to investigate interactions between treatment and conditions, repeated measures analyses of variance were conducted with mean *Z*-values of the insula, inferior frontal gyrus and amygdala as dependent variables, context (sick versus control sound, bored versus control sound) and frequency (500 Hz, 700 Hz) as within-subject factors, and treatment (oxytocin, placebo) as between-subject factor. Use of oral contraceptives and menstrual cycle were included as between-subject factors in the analyses in order to control for influences of these variables. The mode of menstrual cycle (luteal phase) was assigned to the two participants with unknown menstrual cycle phase.

Results

In a series of repeated measures analyses of variance we tested treatment (oxytocin versus placebo) and context effects (bored versus sick) on a priori selected brain areas: the insula, inferior frontal gyrus, and amygdala. For the results of the whole brain analysis see Supplementary Material (Figure s1).

Insula. The repeated measures analysis of variance with insula activation as dependent variable showed a significant main effect of context ($F(1,42) = 10.32$, $p < .01$, partial $\eta^2 = .20$). There was increased activation in the insula during exposure to crying of a sick infant (versus control sound), but decreased activation during exposure to crying of a bored infant (versus control sound), see Figure 2. This main effect was qualified by a significant interaction between treatment and context ($F(1,42) = 7.06$, $p = .01$, partial $\eta^2 = .14$). The effect of context was significant in the oxytocin group ($F(1,22) = 19.75$, $p < .001$, partial $\eta^2 = .47$), increasing insula activation during exposure to crying of a sick infant, and decreasing activation during exposure to crying of a bored infant (see Figure 3), whereas in the placebo group there was no significant difference between the sick and bored condition ($F(1,20) = 0.14$, $p = .72$, partial $\eta^2 = .01$). There were no significant main effects of treatment ($F(1,42) = 0.43$, $p = .52$) and frequency ($F(1,42) = 0.18$, $p = .68$). Neither were there significant interactions between treatment and frequency ($F(1,42) = 1.04$, $p = .31$), frequency and context ($F(1,42) = 2.95$, $p = .09$) or between treatment, frequency, and context ($F(1,42) = 1.65$, $p = .21$).

Inferior frontal gyrus. The repeated measures analysis of variance with IFG activation as dependent variable revealed a significant effect of context ($F(1,42) = 9.53$, $p < .01$, partial $\eta^2 = .19$). The IFG was significantly more activated during exposure to crying of a sick infant (versus control sound) compared with crying of a bored infant (versus control sound), but this main effect was qualified by a significant interaction between treatment and context ($F(1,42) = 7.09$, $p = .01$, partial $\eta^2 = .14$). The effect of context was highly significant in the oxytocin group ($F(1,22) = 24.24$, $p < .001$, partial $\eta^2 = .52$), but there was no significant difference between the sick and bored condition in the placebo group ($F(1,20) = 0.07$, $p = .80$, partial $\eta^2 = .00$). Again, oxytocin increased activation in the IFG during exposure to crying of a sick infant, but decreased activation during exposure to crying

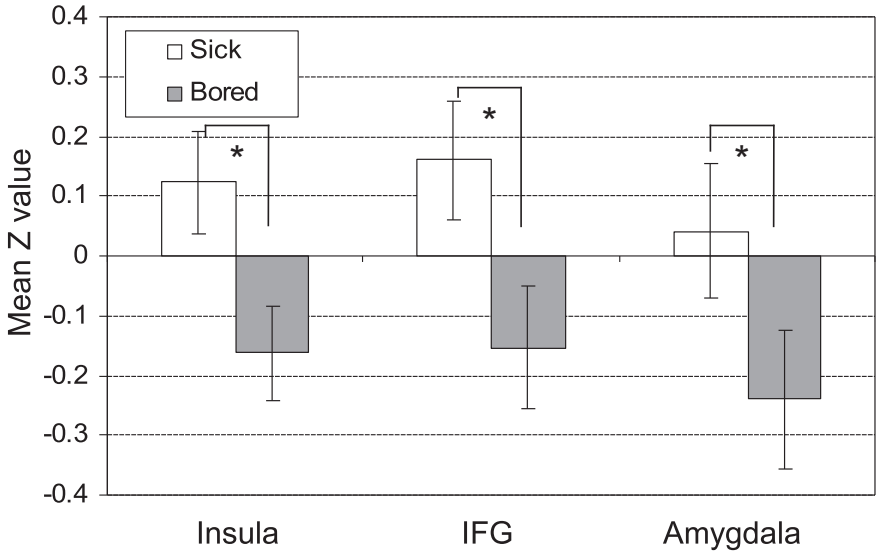

Figure 2. Z-values (*M, SE*) of left insula, left inferior frontal gyrus (IFG), and right amygdala activation during crying of a sick infant (versus control sound, 500 and 700 Hz) and crying of a bored infant (versus control sounds, 500 and 700 Hz). $n = 50$, $^*p < .01$

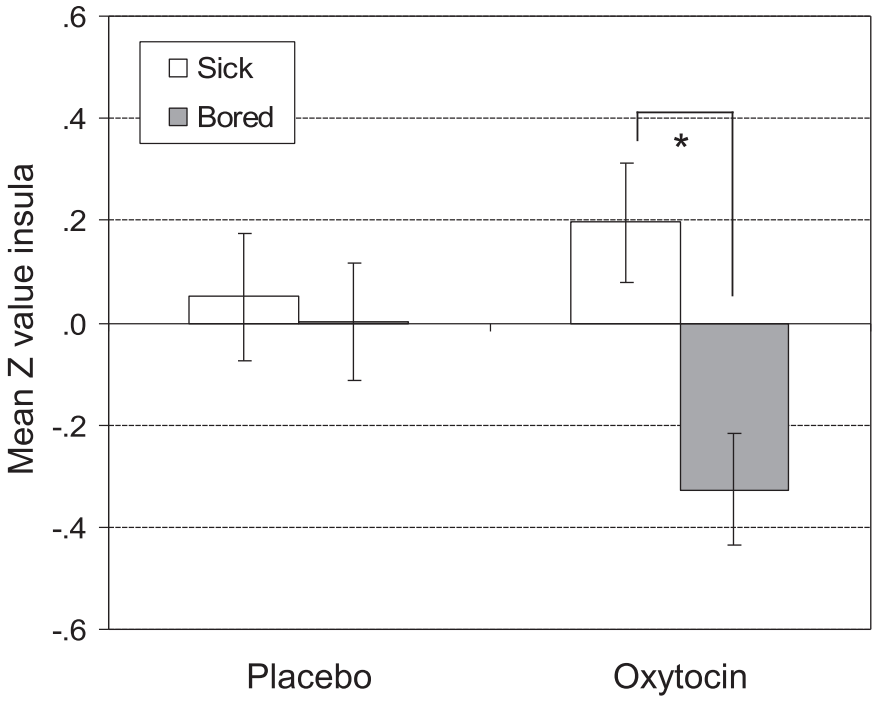

Figure 3. Z-values (*M, SE*) of left insula activation during crying of a sick infant compared with control sounds (500 and 700 Hz) and crying of a bored infant compared with control sounds (500 and 700 Hz) for individuals in the oxytocin and placebo condition. $^*p < .001$

of a bored infant, with similar effects absent in the placebo group, see Figure 4. There were no significant main effects of treatment ($F(1,42) = 0.01$, $p = .94$) and frequency ($F(1,42) = 0.09$, $p = .76$) and no interaction between treatment and frequency ($F(1,42) = 0.78$, $p = .38$), frequency and context ($F(1,42) = 1.00$, $p = .32$), or between treatment, context, and frequency ($F(1,42) = 0.23$, $p = .64$).

Amygdala. The repeated measures analysis of variance with amygdala activation as dependent variable showed a significant effect of context ($F(1,42) = 8.01$, $p < .01$, partial $\eta^2 = .16$). There was increased activation in the amygdala during exposure to crying of a sick infant (versus control sound), but decreased activation during exposure to crying of a bored infant (versus control sound), see Figure 2. Again, the effect of context was only significant in the oxytocin group ($F(1,22) = 6.70$, $p = .02$, partial $\eta^2 = .23$) and not significant in the placebo group ($F(1,20) = 1.81$, $p = .19$, partial $\eta^2 = .08$), but the interaction between treatment and context was not significant ($F(1,42) = 1.42$, $p = .24$). However, there was a significant interaction between treatment and frequency ($F(1,42) = 7.20$, $p = .01$, partial $\eta^2 = .15$). Oxytocin significantly decreased amygdala activation during exposure to 500 Hz crying, but increased amygdala activation during exposure to 700 Hz crying, with similar effects absent in the placebo group (see Figure 5). There were no significant main effects of frequency ($F(1,42) = 1.24$, $p = .27$) and treatment ($F(1,42) = 0.10$, $p = .76$). Neither were there significant interactions between frequency and context ($F(1,42) = 1.74$, $p = .20$) or treatment, context, and frequency ($F(1,42) = 1.31$, $p = .26$).

We examined the potential moderating influence of experiences with parental rejection but results were not significant (data available on request).

Discussion

In this study we examined the influence of oxytocin on neural responses to crying that was indicated as coming from a sick infant and crying coming from a bored infant. We found that intranasally administered oxytocin led to more pronounced differences in neural responding to sick infant crying compared with bored infant crying, indicating enhanced processing of the contextual information. Oxytocin significantly increased insula and IFG responding to crying of a sick infant, but decreased activation in these brain regions during exposure to crying of an infant that was labeled as bored. In addition, we found that oxytocin decreased amygdala responding to crying at 500 Hz, but increased amygdala responding to crying at 700 Hz. These findings indicate that oxytocin enhances the salience of the context and of the acoustics of crying, thus facilitating the interpretation of the infant's crying and the selection of an adequate caregiving response.

Infant crying has often been described as a paradoxical signal (Soltis, 2004). It enhances infant survival by eliciting care and by conveying information on the health condition of the child, but also evokes aversive and angry feelings in parents and can trigger child abuse and neglect (Barr, Trent, & Cross, 2006). The likelihood of using such harsh caregiving responses is increased when infants are sick, possibly because sick, high-pitched crying is perceived as more aversive (Soltis, 2004). Consistent with previous results (Riem et al., 2011), we found that

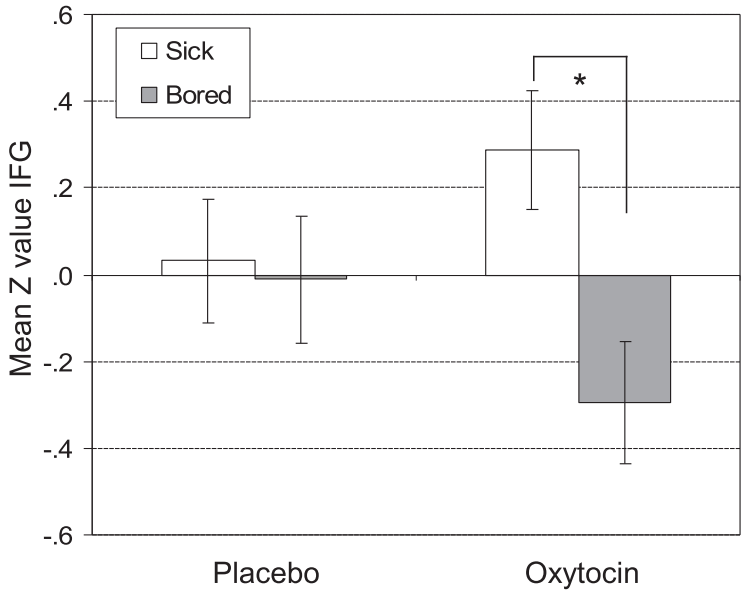

Figure 4. Z-values (*M, SE*) of left inferior frontal gyrus (IFG) activation during crying of a sick infant compared with control sounds (500 and 700 Hz) and crying of a bored infant compared with control sounds (500 and 700 Hz) for individuals in the oxytocin and placebo condition. *$p < .001$

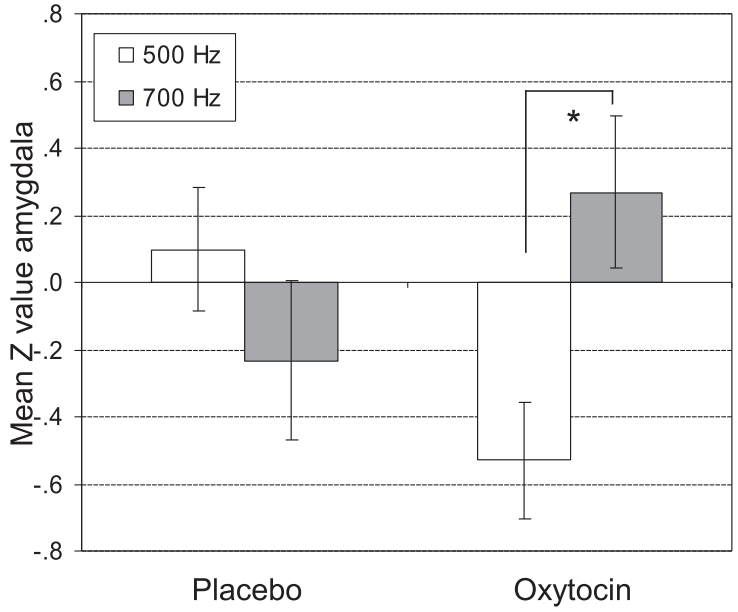

Figure 5. Z-values (*M, SE*) of right amygdala activation during crying (sick and bored infants) at 500 Hz compared with control sounds and crying (sick and bored infants) at 700 Hz compared with control sounds for individuals in the oxytocin and placebo condition. *$p < .01$

oxytocin increased activation in empathy-related brain regions during exposure to sick infant crying, thus facilitating prompt and sensitive responses to sick infants' crying. Increased empathic feelings may enhance the motivation to alleviate the infant's distress in a sensitive way, and decrease harsh caregiving responses that are only aimed at stopping the infant's crying because it is aversive.

Whereas sick infant crying requires a prompt response, a more delayed response may be adequate in case of mild distress, such as fussiness due to boredom. Hubbard and Van IJzendoorn (1991) found that delay of maternal response reduced the number of crying bouts during the first half year of life. The concept of differential responsiveness, which implies that mild distress should not be interpreted in terms of the infant's attachment needs, and that only severe distress requires a very prompt response, may explain these findings. A delayed response to a fussy infant might even be more sensitive because it enables the infant to learn to cope with situations of mild distress. Interestingly, we found that oxytocin reduced activation in the insula and IFG during exposure to bored infant crying. This may indicate that oxytocin reduces concern and empathic feelings for a bored infant, thereby lowering the perceived urgency of this type of crying and stimulating a delayed (in that context sensitive) response to infant fussiness.

Furthermore, we found that oxytocin decreased amygdala responses during exposure to 500 Hz crying, but it increased amygdala responses to 700 Hz crying. This is partly in line with a previous study that showed that oxytocin decreased amygdala activation during exposure to the same infant sounds (and 900 Hz crying) but without context information (Riem et al., 2011). The amygdala is involved in the perception of infant stimuli and has been associated with the experience of fear and the salience of stimuli (Davis & Whalen, 2001; LeDoux, 2000). Several studies have shown that reduced amygdala activation is one of the mechanisms underlying the anxiolytic effects of oxytocin (Domes et al., 2007; Kirsch et al., 2005). Under normal circumstances, reduced amygdala activation might promote sensitive responding to crying by preventing parents from being overwhelmed by negative emotions (Riem et al., 2011). However, reduced levels of arousal might be maladaptive in the case of high-pitched crying, since this type of crying generally signals that the infant is sick or in pain. The finding that oxytocin enhances amygdala responding to high-pitched crying might reflect increased vigilance to signals indicating that the infant is in danger. This interpretation is consistent with a study by Domes et al. (2010) who found that oxytocin enhanced amygdala reactivity to fearful and angry faces in women, thus facilitating the detection of threat signals and triggering reactions to protect the child from adult strangers with frightening faces.

The limitations of our study should be acknowledged. First, we used a between-subjects design to study the effects of oxytocin, which implies the risk of pre-existing differences between the oxytocin and placebo group that might have influenced the results. Randomization and double-blind application have decreased this risk substantially. Moreover, meta-analytic findings indicate no significant differences in outcomes of studies using between- or within-subject designs to examine intranasal oxytocin influences (Van IJzendoorn & Bakermans-

Kranenburg, 2012). Second, the results of this study can only be generalized to women without children. Furthermore, we did not include neural responses to infant crying without context information, which makes it difficult to relate the present findings with the results of our previous study (Riem et al., 2011).

In sum, the findings of our study indicate that the effects of oxytocin are dependent on perceived context, which is in line with previous research showing that the neural and behavioral effects of oxytocin are shaped by social context (Bartz et al., 2011). We found that oxytocin enhanced insula, IFG and amygdala responding to sick or high-pitched infant crying compared with crying of a bored infant, thus enhancing vigilance to signals indicating that the infant is in danger. This is the first study to show that intranasal administration of oxytocin leads to more pronounced differences in neural responding to sick infant crying compared to bored infant crying. Our findings indicate that oxytocin enhances the processing of contextual cues and the acoustics of crying, thus facilitating sensitive responding to infant crying in various manifestations.

Supplementary material to: Pity or Peanuts? Oxytocin affects neural response to sick and bored infant crying

fMRI data acquisition. Cushions were placed between the head coil and the participant to prevent head movement. All anatomical scans were examined by a radiologist and no anomalous findings were reported.

Sounds. 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. A new 10-sec cry sound with overall Peak F0 of 714.5 Hz (700 Hz cry) was created by digitally increasing the pitch of the original cry. 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.

Whole brain analysis. The contrasts sick 500 Hz > control 500 Hz, sick 700 Hz > control 700 Hz, bored 500 Hz > control 500 Hz, and bored 700 Hz > control 700 Hz revealed significant activation in brain regions involved in auditory information processing, including the bilateral superior temporal gyrus, planum temporale, and Heschl's gyri in the oxytocin and placebo group (see Figure s1 for activations in the placebo group). There were no significant differences in activation between the sick 500 Hz and bored 500 Hz conditions or between the sick 700 Hz and bored 700 Hz conditions in the oxytocin and placebo group. Furthermore, there were no significant effects of oxytocin on activation during sick infant crying compared with control sounds at 500 or 700 Hz, bored infant crying compared with control sounds at 500 or 700 Hz, or during sick infant crying compared with bored infant crying at 500 or 700 Hz.

Mood. Participants rated on five-point Likert scales how much irritation, fear, enthusiasm, and alertness they felt. We conducted repeated measures analyses of variance with reported irritation, fear, enthusiasm, and alertness as dependent variables, treatment (oxytocin and placebo) as between-subject factor and time (time 1: before drug administration, and time 2: after scanning) as within-subject factor to test for effects of oxytocin on self-reported mood. There were no significant effects of treatment (ps > .09) and no significant interactions between treatment and time (ps > .33).

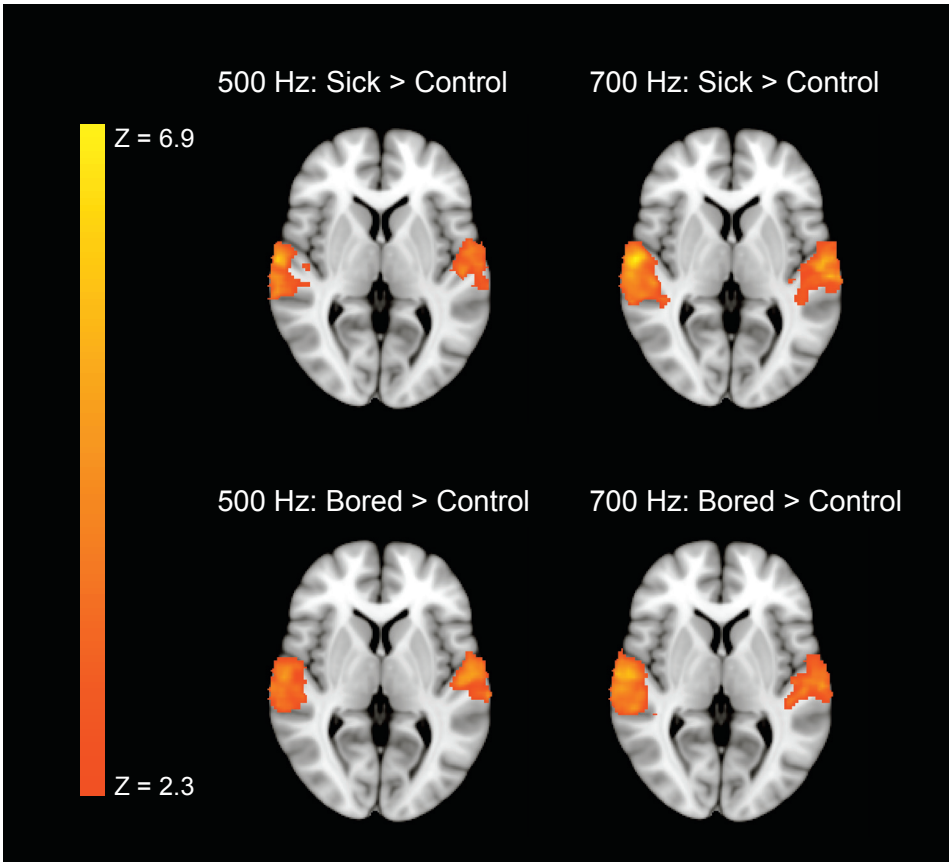

Figure S1. Significant activation in the bilateral superior, planum temporale and Heschl's gyri for the contrasts Bored > Control and Sick > Control at 500 and 700 Hz in the placebo group. Statistical images were thresholded with clusters determined by Z > 2.3 and a cluster-corrected significance threshold of $p < .05$. The right side of the brain corresponds to the left hemisphere and vice versa, z = 4.

6 Does intranasal oxytocin promote prosocial behavior to an excluded fellow player? A randomized-controlled trial with Cyberball

Madelon M.E. Riem, Marian J. Bakermans-Kranenburg, Renske Huffmeijer, & Marinus H. van IJzendoorn, (in press). Psychoneuroendocrinology.

Abstract

The neuropeptide oxytocin has been shown to stimulate prosocial behavior. However, recent studies indicate that adverse early caregiving experiences may moderate the positive effects of oxytocin. In this double blind randomized-controlled trial we investigated the effects of oxytocin on prosocial behavior during a virtual ball-tossing game called Cyberball. We examined the influence of oxytocin on prosocial helping behavior toward a socially excluded person who was known to the participant, taking into account early caregiving experiences and the emotional facial expression of the excluded person as potential moderators. Participants were 54 women who received a nasal spray containing either 16 IU of oxytocin or a placebo and had 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 participants compensated for other players' ostracism by throwing the ball more often toward the excluded player. Oxytocin administration further increased the number of ball throws toward the excluded person, but only in individuals who experienced low levels of maternal love withdrawal. The facial expression of the excluded person did not affect prosocial helping behavior and did not moderate the effects of oxytocin. Our findings indicate that the positive effects of oxytocin on prosocial behavior toward a victim of social exclusion are limited to individuals with supportive family backgrounds.

Introduction

Ostracism, the exclusion of an individual by other group members, induces strong negative emotions. Several studies have examined the effects of being ostracized with a virtual ball-tossing game called Cyberball (Williams & Jarvis, 2006). Being excluded during Cyberball results in lower levels of feelings of belonging, control, and meaningful existence (Eisenberger & Lieberman, 2004; Eisenberger, Lieberman, & Williams, 2003; Gonsalkorale & Williams, 2007; Zadro, Williams, & Richardson, 2004), and emotional responses such as aggression (Chen, DeWall, Poon, & Chen., 2012), anger (Chow, Tiedens, & Govan, 2008), and jealousy (Harmon-Jones, Peterson, & Harris, 2009). In addition, exclusion during Cyberball has been associated with activation of a neural pain network consisting of brain regions involved in bodily injury as well as social pain (Eisenberger, Jarcho, Lieberman, & Naliboff, 2006).

Although many studies used Cyberball to study the effects of being excluded, few studies investigated the way individuals respond when they observe someone else being excluded. Observing someone being ostracized during Cyberball confronts the participant with a dilemma: he or she can help the excluded person by throwing the ball more often to the victim, or he or she can go along with the crowd and also exclude the victim (Williams & Jarvis, 2006). The latter might be the safest option, because helping an excluded person participants is facing the risk of being excluded yourself. Beeney, Franklin, Levy, and Adams (2011) found that the neural pain network involved in social pain is similarly activated when participants see someone else suffering social rejection during Cyberball or when they suffer exclusion themselves, especially when they know the ostracized person. Observing someone else being ostracized however also activates brain regions involved in empathy (Masten, Eisenberger, Pfeifer, & Dapretto, 2010). Masten, Morelli and Eisenberger (2011) showed that activation in empathy-related brain regions was associated with later prosocial behavior toward the victim, indicating that individuals who feel more empathy for a person in distress will make greater efforts to help the victim. However, their study focused on subsequent prosocial behavior, operationalized as sending prosocial emails to the victim, and it is not yet known whether individuals show prosocial helping behavior toward the victim *during* social exclusion.

A number of studies have shown that prosocial behavior is enhanced by the neuropeptide oxytocin (Insel, 2010; Van IJzendoorn & Bakermans-Kranenburg, 2012). Oxytocin is involved in mother-infant bonding, sensitive parenting, and the perception of infant signals (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Riem et al., 2011; Riem, Pieper, Out, Bakermans-Kranenburg, & Van IJzendoorn, 2011). Studies have shown that intranasal administration of oxytocin promotes a range of social behaviors, including trust (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), in-group altruism (De Dreu et al., 2010), empathy (Bartz, Zaki, Bolger, et al., 2010) and sensitivity to infant signals (Naber, Van IJzendoorn, Deschamps, Van Engeland, & Bakermans-Kranenburg, 2010; Riem et al., 2012). In addition, it has been shown that intranasal oxytocin influences social behavior during the Cyberball game in individuals

with autism (Andari et al., 2010). Participants played the ball-tossing game with three fictitious players with different cooperative profiles (good, bad, neutral). After oxytocin administration, participants with autism responded more strongly to the most socially cooperative partner, indicating that oxytocin enhanced their ability to process socially relevant cues.

However, oxytocin might not enhance social behavior similarly for all people. Contextual and individual differences seem to moderate oxytocin effects on social behavior and cognition (Bartz, Zaki, Bolger, & Ochsner, 2011; Van IJzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, & Tops, 2011; Bakermans-Kranenburg, Van IJzendoorn, Riem, Tops, & Alink, 2012). Bartz, Zaki, Ochsner, et al. (2010) found that effects of oxytocin administration on childhood memories were moderated by participants' attachment representations. Less anxious individuals remembered their mother as more caring and close after oxytocin (vs. placebo) administration, whereas more anxiously attached individuals remembered their relationship with their mother in a more negative light in the oxytocin (vs. placebo) condition. Furthermore, De Dreu et al. (2010) found that intranasal oxytocin enhanced in-group altruism, but at the same time increased defensive reactions toward out-group members. Thus, oxytocin may drive a 'tend and defend' response.

Others studies found that the beneficial effects of oxytocin on prosocial behavior are moderated by harsh caregiving experiences. Intranasal oxytocin decreased the use of excessive handgrip force in response to infant crying, but only in individuals with supportive family backgrounds (Bakermans-Kranenburg et al., 2012). Similarly, Van IJzendoorn et al. (2011) found that oxytocin administration increased participants' willingness to donate money to a charity, but only in participants who experienced low levels of parental love withdrawal, a parental disciplinary strategy that involves withholding love and affection when a child misbehaves or fails at a task. Moreover, effects of oxytocin on complex brain networks involved in self-referential processing and affectionate touch were moderated by experiences of maternal love withdrawal, indicating that unsupportive caregiving experiences also suppress the effects of oxytocin at the neural level (Riem et al., in press). Love withdrawal is considered psychological maltreatment when used excessively (Euser, Van IJzendoorn, Prinzie, & Bakermans-Kranenburg, 2010) and has been related to high concern over mistakes, low emotional well-being and feelings of rejection and resentment toward the parents (Elliot & Thrash, 2004; Goldstein & Heaven, 2000; Renk, McKinney, Klein, & Oliveros, 2006). These negative emotions may hinder empathic responses and prosocial helping behaviors toward victims of social exclusion.

To our knowledge, this is the first randomized-controlled trial investigating the effects of oxytocin administration on prosocial behavior during Cyberball. Whereas previous studies used Cyberball to study the effects of being socially excluded, we are the first to examine individuals' responses when they see someone else being excluded. We examined the influence of oxytocin on prosocial helping behavior toward an excluded person who was known to the participant, namely the experimenter. In addition, we examined whether the effects of oxytocin were

dependent on maternal use of love withdrawal and on the facial expression of the excluded experimenter. We expected that oxytocin administration would increase the number of ball throws to the excluded experimenter, but only in individuals who experienced low levels of maternal love withdrawal. Furthermore, as sad facial expressions might elicit more empathic feelings compared with neutral facial expressions, we expected larger increases in the number of ball throws to the excluded experimenter when she showed a sad facial expression compared to the excluded experimenter with a neutral facial expression.

Method

Participants

A total of 343 female undergraduate students from the departments of education and child studies, and psychology at Leiden University participated in the first phase of the study. In this phase, the participants completed online questionnaires on their perception of parenting by their mothers, and some demographic details. One participant was excluded due to random responses. Five females with children of their own were also excluded. One hundred eighty six students participated in the second phase of the study, which was designed to examine behavioral and physiological responding to infant crying. Fifty participants with scores ranging from low to high on a questionnaire on parenting were selected to participate in the third phase of the study, consisting of an fMRI study and the Cyberball task. Participants were randomly assigned to the oxytocin or the placebo condition. Four additional participants were selected because of problems with fMRI data acquisition, resulting in a total sample size of 54 participants for the current study (28 participants in the oxytocin condition and 26 participants in the placebo condition). Participants were screened for MRI contraindications, psychiatric or neurological disorders, hearing problems, pregnancy, and alcohol and drug abuse. The mean age of the participants was 19.63 years (*SD* =1.43, range 18-27). The majority (72.2 %) used oral contraceptives. Permission for this study was obtained from the Ethics Committees of the Institute of Education and Child Studies of Leiden University and of the Leiden University Medical Center. The results of the fMRI study will be reported elsewhere.

Procedure

Participants were invited for Cyberball preferably in the luteal phase of their menstrual cycle in order to control for influences of menstrual cycle. During the luteal phase, plasma oxytocin levels are lower (Salonia et al, 2005) and more responsive to stimulation such as by nipple stimulation (Leake et al, 1984). Therefore, effects of oxytocin nasal administration might be more pronounced during the luteal phase. Approximately 90 min before the start of the Cyberball task participants took 6 puffs of nasal spray containing of oxytocin (16 IU total) or 6 puffs of a placebo-spray under supervision of the experimenter. Salivary oxytocin levels have been shown to remain strongly elevated in a stable way up to at least 2¼ after administration of nasal spray containing 16 IU of oxytocin (Huffmeijer et al., 2012) and effects of 16 IU of oxytocin on social behavior and

neural activity have been reported in previous studies (Bakermans-Kranenburg et al., 2012; Riem et al., 2011; Riem et al., 2012). Drug administration was double-blind. Participants were led to believe that they were playing an online ball-toss game called Cyberball (Williams & Jarvis, 2006) with the experimenter who gave instructions during the first part of the lab session (the fMRI study) and two other unknown female individuals. After fMRI data acquisition participants were told by the experimenter that they were going to play a game over the internet and they were introduced to a second experimenter who set up the Cyberball task. The participants were told that the experimenter and the two other players were playing the game in other rooms.

The Cyberball task

The Cyberball game was an adapted version of the task that was used in the study by Crowley, Wu Molfese, and Mayes (2010). The participants' glove was at the bottom of the screen. The gloves, pictures, and names of the unknown players were to the left and right of the screen center, and the glove, name, and picture of the experimenter with a neutral or sad expression were at the upper part of the screen, see Figure 1. The experimenter was a 27-year-old female, similar to the average participant and the two other players and the same person for all participants. The experimenter was selected as the known person in order to control for differences in familiarity of the known person among participants. Participants were instructed to throw the ball to the other players using the keyboard. The game consisted of three blocks of 48 trials each. The first block was a fair situation in which all players received one fourth of the throws. In the second and third block, the experimenter was excluded from the game and did not receive any throws from the two unknown players. In the third block, the facial expression of the excluded experimenter changed from neutral to sad in the sad condition, but it did not change in the neutral condition. The sad facial expression did not change when the participant threw the ball to the ostracized experimenter. Participants played the entire game (fair play block, unfair play block 1, unfair play block 2) twice: once with the experimenter with a neutral expression and once with the experimenter with a sad expression. There was a short break between the sad and the neutral condition and the order of neutral and sad conditions was counterbalanced across participants. There were small differences in the total number of total throws of the participants. Therefore, we calculated the ratio of throws of the participant to the experimenter by dividing the number of throws of the participant to the experimenter by the total number of throws by the participant to any of the players. A ratio larger than .33 in the unfair play block indicates that participants compensate for the other players' ostracism by throwing the ball more often to the excluded experimenter. Ratios were calculated for each play block. We calculated ratios for the sad and neutral condition separately as well as the ratio of ball throws to the known player in the sad and neutral condition together, independent of emotion. One of our questions was whether the effect of oxytocin on prosocial behavior depended on facial expression of the excluded player, which did not change before the third block. Therefore, unfair play block 1 was excluded from the analysis on the

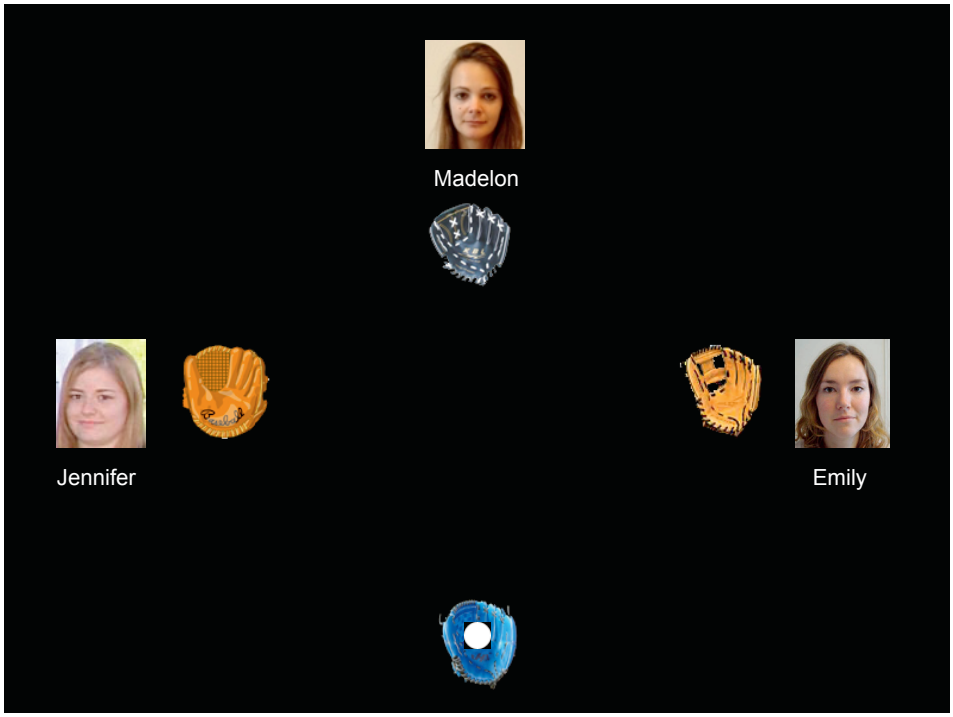

Figure 1. Set-up Cyberball task in the neutral condition. The participants' glove was at the bottom of the screen. The gloves, pictures and names of the unknown players were to the left and right of the screen center. The glove, name and picture of the experimenter with a neutral or sad expression were at the upper part of the screen.

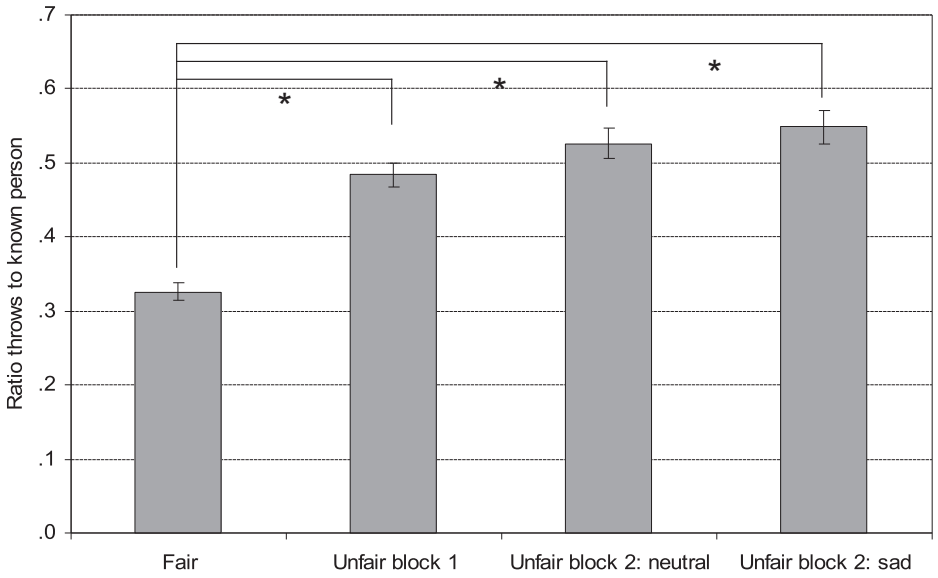

Figure 2. Ratio of throws (*M, SE*) to the known player in the fair play block, unfair play block 1, and unfair play block 2 with the neutral and the sad experimenter. * $p < .001$

effects of oxytocin on prosocial responding to the excluded experimenter with a sad or neutral expression.

Maternal love withdrawal

The questionnaire on maternal use of love withdrawal contained 8 items of the Withdrawal of Relations subscale of the Children's Report of Parental Behavior Inventory (CRPBI; Beyers and Goossens, 2003; Schludermann and Schludermann, 1983). Because it is in particular the use of *maternal* love withdrawal that has been related to low emotional well-being in adolescence and adulthood (Elliot & Thrash, 2004; Renk, McKinney, Klein, & Oliveros, 2006), we focused on maternal caregiving only. The questionnaire was completed online during the first phase of the study. Participants rated how well each of the 8 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). The average item score on the love withdrawal questionnaire was 1.68 (SD = 0.77). The scores did not differ for participants in the oxytocin or placebo condition, $t(52) = -0.36$, $p = 0.72$. Love withdrawal was dichotomized into low versus high love withdrawal using a median split (median = 1.40). In the placebo group, 12 participants reported low levels of love withdrawal and 14 participants reported high levels of love withdrawal. In the oxytocin group, 15 participants reported low levels of love withdrawal and 13 participants reported high levels of love withdrawal.

Results

To examine whether participants compensated for the other players' ostracism by throwing the ball more often to the excluded player, a repeated measures analysis was performed with the ratio of ball throws to the known player as dependent variable and play block (fair play blocks, unfair play block 1, unfair play block 2 with neutral experimenter, unfair play block 2 with sad experimenter) as within-subject variable. There was a significant effect of play block on throws to the experimenter ($F(2.54,134.60) = 50.70$, $p < .001$). Contrasts indicated that participants threw the ball more often to the excluded experimenter in unfair play block 1 ($F(1,53) = 89.54$, $p < .001$) compared with the fair play block. In addition, participants threw the ball significantly more often to both the neutral and sad experimenter in the unfair play block 2 compared with the fair play block (neutral: $F(1,53) = 98.33$, $p < .001$; sad: $F(1,53) = 97.80$, $p < .001$) (see Figure 2). There was no significant difference between the neutral and sad condition in the unfair play block 2 ($F(1,53) = 0.86$, $p = .36$).

In order to examine the effects of love withdrawal on prosocial helping behavior under natural circumstances, a repeated-measures analysis was conducted with ratio of ball throws to the known player as dependent variable, emotion (excluded experimenter with neutral *versus* sad facial expression) as within-subject variable and love withdrawal (low *versus* high) as between-subject factor for participants in the placebo group. Participants with high levels of love withdrawal tended to be more prosocial than participants with low levels of

love withdrawal, but the difference was not significant ($F(1,24) = 3.53$, $p = .07$). Furthermore, there was no significant difference between ratio throws to the excluded experimenter with a neutral and sad facial expression ($F(1,24) = 0.79$, $p = .38$) and no significant interaction between love withdrawal and facial expression ($F(1,24) = 0.11$, $p = .75$).

In order to examine oxytocin effects during the Cyberball task on prosocial behavior depending on emotional expression and love withdrawal we conducted a repeated-measures analysis with the ratio of ball throws to the known player in the unfair condition as dependent variable, emotion (neutral, sad) as within-subject factor and nasal spray group (oxytocin, placebo) and love withdrawal group (low versus high love withdrawal) as between-subject factors. There were no significant main effects of emotion ($F(1,50) = 0.81$, $p = .37$), nasal spray group ($F(1,50) = 0.56$, $p = .46$) and love withdrawal group ($F(1,50) = 0.00$, $p = .97$). Neither were there significant interactions between emotion and nasal spray group ($F(1,50) = 0.05$, $p = .83$) or between emotion and love withdrawal group ($F(1,50) = 0.10$, $p = .76$). However, there was a significant interaction between nasal spray and love withdrawal ($F(1,50) = 6.78$, $p = .01$, partial $\eta^2 = .12$). The interaction between nasal spray and love withdrawal was also significant when the fair condition was included in the repeated measures analysis as the first measurement of the within subject factor play block (fair play blocks, unfair play block with neutral experimenter, unfair play block with sad experimenter) ($F(1,50) = 4.52$, $p = .04$, partial $\eta^2 = .08$). Including order of emotion condition (neutral experimenter in first round or sad experimenter in first round) as covariate did not change the significance of the interaction between love withdrawal and nasal spray group ($F(1,49) = 6.59$, $p = .01$, partial $\eta^2 = .12$) and there was no main effect of order ($F(1,49) = 0.47$, $p = .50$). In addition, a hierarchical regression analysis was conducted with ratio of throws as outcome measure, nasal spray group and continuous scores on love withdrawal (centered) in the first step and the interaction between nasal spray and love withdrawal in the second step. The model was not significant ($F(3,50) = 2.00$, $p = 0.13$) and there were no significant effects of nasal spray group (ß = -0.10, $p = 0.46$) and love withdrawal (ß = -0.00, $p = 0.99$). However, the effects of oxytocin were significantly moderated by love withdrawal (ß = 0.31, $p = 0.03$); and this was also the case when love withdrawal was dichotomized into a low love withdrawal group consisting of the 60% lowest scores versus a high love withdrawal group consisting of the 40% highest scores ($F(1,50) = 5.27$, $p = .026$, partial $\eta^2 = .10$), showing the robustness of the interaction effect. Participants threw the ball more often to the excluded player when they were administered oxytocin, but only when they had experienced low levels of love withdrawal. To examine the group differences in mean ratio of ball throws to the excluded player (sad and neutral together) 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. A one-way ANOVA with planned contrasts showed that oxytocin significantly increased the number of ball throws in participants with low love withdrawal scores, $t(50) = 2.36$, $p = .02$, but oxytocin did not have a significant effect for participants reporting high love withdrawal, $t(50) = -1.32$, $p = .19$ (see Figure 3).

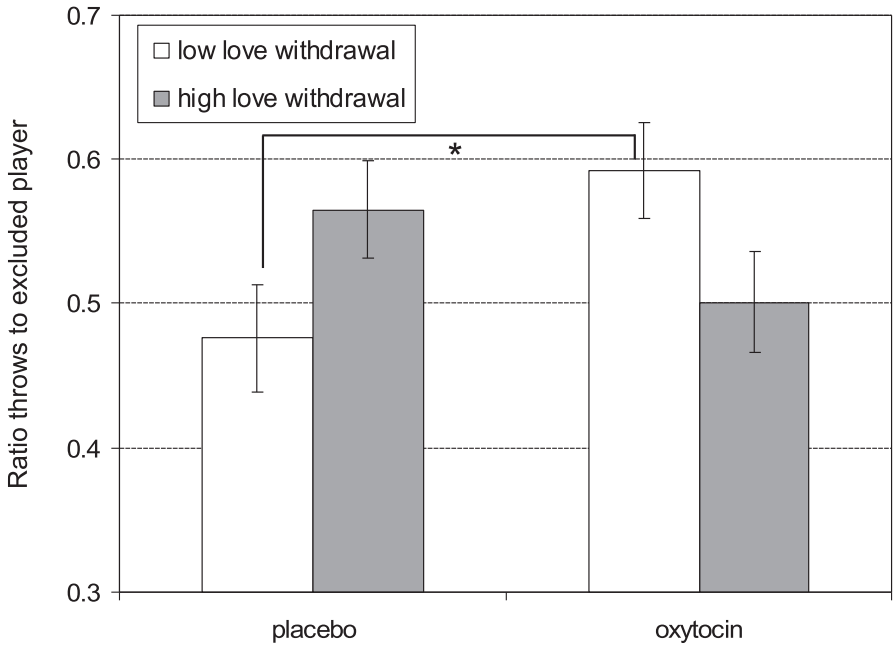

Figure 3. Ratio of throws (*M, SE*) to the excluded player for participants reporting low and high love withdrawal in the placebo and oxytocin group. * $p < .05$

Discussion

In the current study, we examined the influence of intranasal oxytocin administration on prosocial helping behaviors during social exclusion of a player in Cyberball. This study is the first to demonstrate that participants compensate for other players' ostracism by passing the ball more often toward an excluded player who is known to the participant, indicating that individuals show prosocial helping behavior toward a victim of the social exclusion. In addition, we found that oxytocin further increased the number of ball throws toward the excluded person, but only in individuals who experienced a supportive rearing environment. Our findings indicate that the positive effects of oxytocin on prosocial helping behavior are moderated by adverse caregiving experiences. This is in line with previous studies showing that oxytocin does not enhance prosocial behavior in all people under all circumstances (Bakermans-Kranenburg et al., 2012; Bartz et al., 2011; Van IJzendoorn et al., 2011).

What processes might underlie the positive effects of oxytocin on prosocial helping behaviors toward a socially excluded person? As oxytocin has been shown to enhance empathy (Bartz, Zaki, Bolger, et al., 2010) and mentalizing (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007), one way in which it may enhance prosocial helping behavior in individuals with supportive family background is by increasing empathic feelings and the understanding of the emotions felt by the excluded victim. This is consistent with previous research showing that

oxytocin enhances the ability to process socially relevant cues during Cyberball in participants with autism (Andari et al., 2010). Oxytocin may promote the efficient processing of information in empathy-related and social pain-related brain networks involved in seeing someone else being ostracized (Beeney et al., 2011; Masten, Morelli, & Eisenberger, 2011), resulting in more efforts to help the victim.

However, according to Carter (1998), oxytocin promotes social affiliation not only by enhancing social approach-related behavior but also by reducing feelings of anxiety and fear of novelty, which is supported by studies showing that oxytocin has anxiolytic properties (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003). These anxiolytic effects may be mediated by the inhibitory influence of oxytocin on the amygdala (Gamer, Zurowski, & Buchel, 2010; Kirsch et al., 2005; Riem et al., 2011), a brain region involved in fear processing (MacLean, 1990). Helping a victim of social exclusion can be risky and may elicit anxious feelings since individuals who help an ostracized person face the risk of being excluded themselves (Kanetsuna & Smith, 2002; Latane & Nida, 1981) Therefore, another process explaining our findings may be that oxytocin decreases anxious feelings and thus increases participants' willingness to accept social risks, which is in line with studies showing that oxytocin increases trust among humans (Kosfeld et al., 2005), even when trust has been breached (Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008).

In our study, the effects of oxytocin were hindered but not altered in individuals who experienced high levels of maternal love withdrawal, as opposed to studies showing negative effects of oxytocin in some conditions (Bartz, Zaki, Ochsner, et al., 2010; De Dreu et al., 2010). Our findings are in line with previous research showing that in individuals with harsh caregiving experiences the beneficiary oxytocin effects are absent at the behavioral (Bakermans-Kranenburg et al., 2012) as well as at the neural level (Riem et al., in press). Fries, Ziegler, Kurian, Jacoris, and Pollak (2005) showed that children who experienced early adversity 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 supportive family. Another study showed 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 (Meinlschmidt & Heim, 2007). Furthermore, Heim et al. (2009) found that women who were exposed to child abuse or neglect showed lower oxytocin concentrations in cerebrospinal fluid. Early adversity may lead to a dysregulation of the oxytocinergic system, possibly by influencing the level of methylation in genetic areas regulating the oxytocinergic system (McGowan et al., 2009; Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010), which might lead to lower oxytocin levels and a decreased sensitivity to intranasal oxytocin.

In contrast to our expectations, the emotional expression of the excluded person did not have a significant effect on prosocial helping behavior. Thus, additional information about the feelings of a socially excluded person does not lead to enhanced helping behavior, possibly because observers already feel the pain of

the victim even if the victim shows a neutral facial expression. Neither were the effects of oxytocin on prosocial behavior moderated by the facial expression of the victim. This latter finding is consistent with previous studies showing that the effects of oxytocin on perception of facial expressions are independent of valence. For example, Domes, Heinrichs, Glascher, et al. (2007) found that oxytocin reduces amygdala activation during exposure to happy, fearful and angry facial expressions, with no significant effect of valence (but see Domes et al., 2010). The authors reasoned that decreased amygdala activation during exposure to both positive and negative stimuli might reflect reduced arousal and ambiguity about the predictive value of social stimuli in general (Hsu, Bhatt, Adolphs, Tranel, & Camerer, 2005). This might motivate the individual to initiate approach behavior in order to encounter the social stimulus. In addition, Hurlemann et al. (2010) found that oxytocin increased emotional empathy in response to both positive and negative social stimuli. Thus, our finding that the effects of oxytocin on prosocial helping behaviors were not moderated by the facial expression of the excluded person seems to converge with previous work demonstrating that valence does not strongly affect oxytocin induced changes in empathic feelings and negative emotional arousal.

Some limitations should be noted. First, the use of a between-subject design implies the risk of pre-existing differences between the placebo and oxytocin group. Randomization and double-blind application have decreased this risk substantially. The use of self-reported maternal love withdrawal is another limitation of our study, and interview assessments or observations of experiences with the parents might yield more valid data. In addition, effects of oxytocin administration on prosocial helping behavior might be different in males and women with children. The results of the current study can only be generalized to women without children. Furthermore, further research is needed to specify the effects of oxytocin on prosocial helping behaviors. Neuro-imaging studies may shed more light on the mechanism underlying oxytocin effects, and may clarify whether oxytocin enhances prosocial behaviors such as social helping by increasing empathy and emotion understanding, by decreasing anxious feelings, or by both processes. Lastly, we found that individuals reporting higher levels of love withdrawal tended to be more prosocial in the placebo condition compared with individuals with low levels of love withdrawal. This trend is in contrast with our expectation that love withdrawal would hinder empathic concerns for others. Alternatively, participants with high levels of love withdrawal experienced negative consequences after misbehaving or failing at a task during childhood. Therefore, they might have thrown the ball more often to the experimenter in order to avoid disapproval by the experimenter. It should be noted, however, that this association was not significant and thus should not be taken for granted before being replicated.

The current study is the first to show that oxytocin increases prosocial helping behavior toward an ostracized individual who is known to the participant. Previous studies focused on prosocial behavior toward strangers (e.g. Van IJzendoorn et al., 2011, Kosfeld et al., 2005). Familiarity might facilitate understanding of the mental state of a person and influence the way the brain responds when

observing that person being socially excluded (Beeney et al., 2011). Moreover, a known person might be considered to be an in-group member, whereas an unknown person might be perceived as an out-group member. The effects of oxytocin tend to be dependent on this in-group *versus* out-group distinction (De Dreu et al, 2010; but see Van IJzendoorn and Bakermans-Kranenburg, 2012). It has been suggested that oxytocin up-regulates behavioral expressions of concern for others more strongly if the other belongs to the in-group (De Dreu, 2012). Thus, it still is unclear whether oxytocin also increases prosocial helping behavior toward an excluded unknown person.

In sum, this study is the first randomized-controlled trial investigating the effects of oxytocin on prosocial helping behavior toward an excluded person during Cyberball. Whereas previous studies used Cyberball to study the effects of being ostracized, our study is the first to examine how individuals respond when they see someone else being ostracized. We found that oxytocin increased prosocial helping behavior toward the excluded person, possibly because of enhanced empathic feelings and understanding of the emotions of the victim and an increased willingness to take social risks. However, the oxytocin induced increases in prosocial helping behavior were only brought about in individuals with supportive family backgrounds. Our findings indicate that the positive effects of oxytocin on social behavior are moderated by early caregiving experiences and provide support for the suggestion that early social adversity can lead to decreased sensitivity to intranasal oxytocin, possibly through methylation of genetic areas regulating the oxytocinergic system.

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

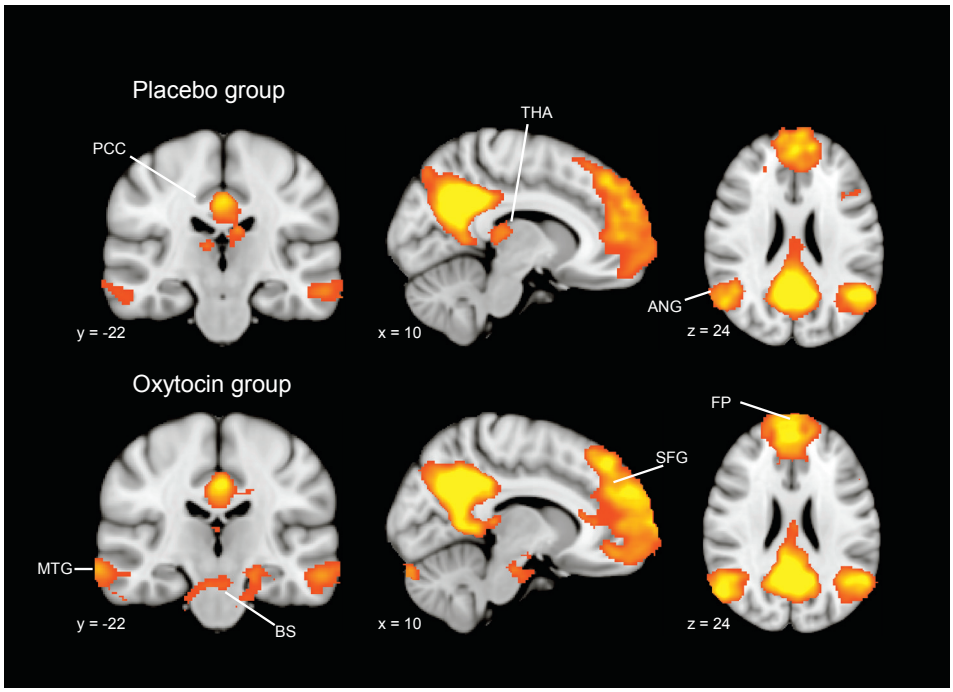

Figure 1. PCC resting-state functional connectivity in the placebo (top panel) and oxytocin group (lower panel), thalamus (THA), angular gyrus (ANG), middle temporal gyrus (MTG), superior frontal gyrus (SFG), frontal pole (FP), brainstem (BS). Statistical images were thresholded with clusters determined 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.

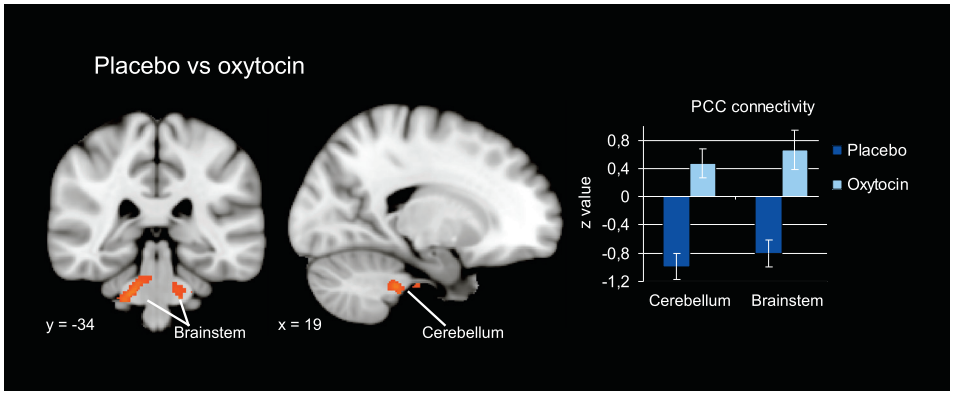

Figure 2. Group difference in PCC resting-state functional connectivity and mean *Z*-values for significantly activated voxels within the brainstem and cerebellum. Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The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

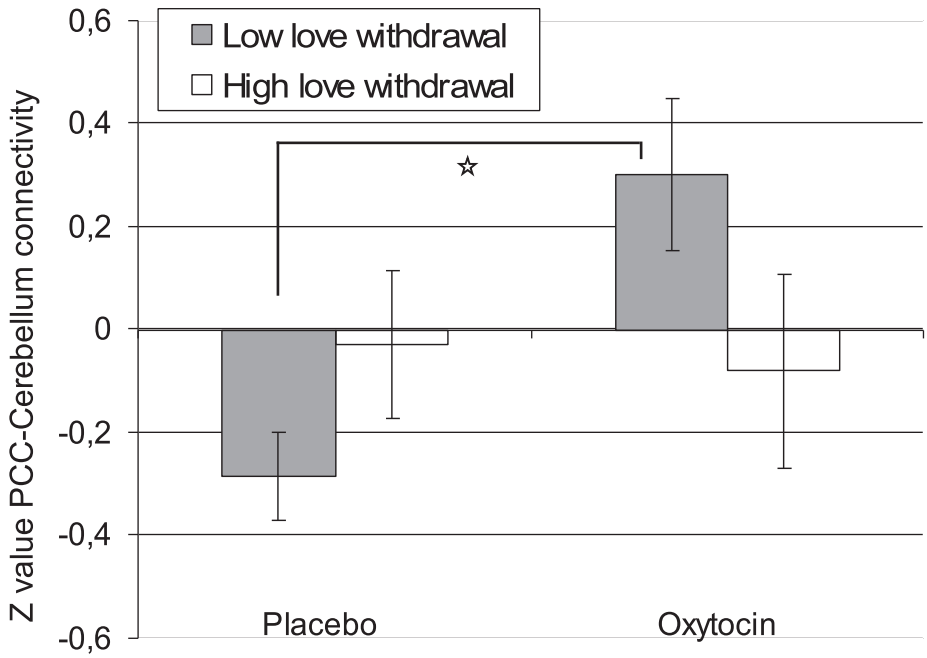

Figure 3 *Z*-values (M, SE) of PCC-cerebellum connectivity for participants reporting low versus high love withdrawal in the placebo group and participants reporting low and high love withdrawal in the oxytocin group. * $p < .05$

& 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.

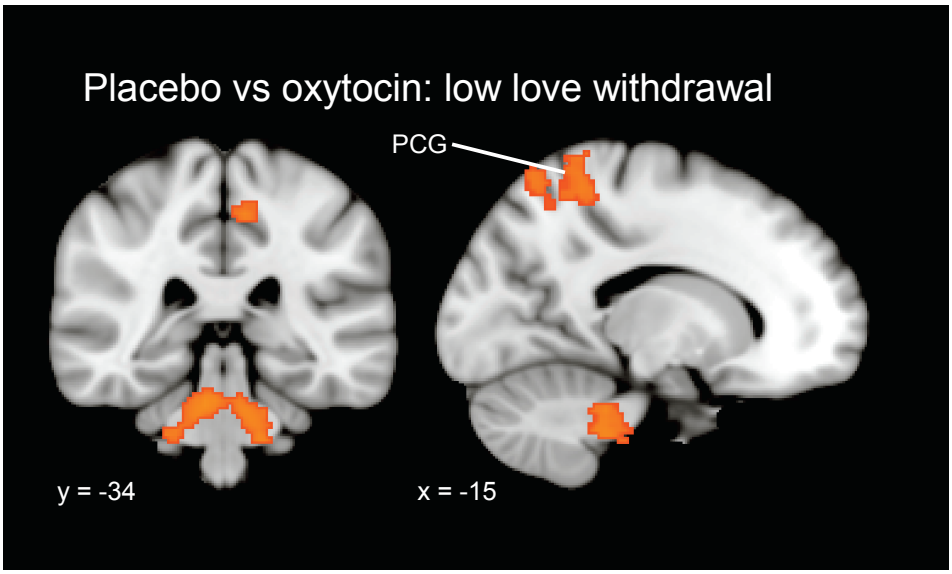

Figure s1. Group difference in PCC resting-state functional connectivity for participants reporting low love withdrawal, postcentral gyrus (PCG). Statistical images were thresholded with clusters determined by $Z > 2.3$ and a cluster-corrected significance threshold of $p < .05$. The right side of the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

8 Discussion

The general aim of the current thesis was to gain more insight into the associations between oxytocin, adult attachment and parenting behaviors, and into the role of context and family background in shaping oxytocin effects. In Chapter 2 and 3, we examined the effects of intranasal oxytocin administration on neural responses to infant crying and laughter in order to clarify the mechanism underlying the putative positive association between oxytocin and parenting. Chapter 4 focused on the influence of adult attachment, measured with the Adult Attachment Interview, on neural, emotional and behavioral responding to infant crying. In Chapter 5, 6 and 7, we examined the role of contextual factors and individuals differences in shaping the effects of oxytocin on social behavior. The findings presented in Chapter 5 indicate that oxytocin has differential effects on neural responding to crying that was indicated as coming from a sick infant and crying coming from a bored infant. In Chapter 6, we show that the effects of oxytocin administration on prosocial behavior during Cyberball are moderated by experiences of love withdrawal. Chapter 7 focused on the effects of oxytocin on functional brain connectivity during rest. The findings presented in this chapter indicate that harsh caregiving experiences moderate the effects of intranasal oxytocin during resting state at the neural level. In the current chapter, these findings will be reviewed in greater detail, followed by a discussion of the limitations, clinical implications and future directions.

Oxytocin and brain responses to infant signals

Oxytocin is well known for its role in lactation, pregnancy, initiation of maternal care, and parenting (Carter, 1998). It was as yet unclear *how* oxytocin affects parenting behaviors. The studies presented in Chapters 2 and 3 were the first to clarify the role of oxytocin in parenting by investigating the influence of intranasally administered oxytocin on neural responses to infant signals. In Chapter 2, oxytocin effects on neural responding to infant crying were investigated. The participants in this study received intranasal oxytocin or a placebo nasal spray and listened to infant crying sounds and control sounds while BOLD responses were measured with fMRI. We found that oxytocin reduced activation in the amygdala, a brain region important for the experience of fear and aversion (LeDoux, 2000), whereas it increased activation in the insula and inferior frontal gyrus (IFG), brain regions involved in empathy and emotion understanding (Decety & Jackson, 2004; Shamay-Tsoory, 2011; Singer, Critchley, & Preuschoff, 2009). The effects of oxytocin might thus be twofold in that it promotes responsiveness to crying by preventing parents from being overwhelmed by anxious or aversive feelings *and* by enhancing empathic and warm feelings for the infant. This is consistent with Carter's (1998) suggestion 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. Indeed, it has been shown that oxytocin has anxiolytic and stress-reducing effects in breastfeeding mothers (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Heinrichs et al., 2001), reduces amygdala responses to fearful social stimuli (Kirsch et al., 2005), and enhances empathy and the understanding of emotions (Bartz et al., 2010; Domes, et al., 2007).

In Chapter 3, we examined the influence of intranasal oxytocin on neural responding to infant laughter. We examined oxytocin effects on functional activation during exposure to infant laughter. In addition, oxytocin effects on functional connectivity were examined, as previous studies have shown that by modulating amygdala activity hormones can shift neural output towards other brain regions within a neural network involved in emotional processing (Bos, Panksepp, Bluthé, & Van Honk, 2012). More specifically, we investigated whether oxytocin affected the functional connectivity between the amygdala, the orbitofrontal cortex (OFC), and anterior cingulate cortex (ACC), brain regions involved in reward processing and emotional regulation (Bos et al., 2012; Pessoa, 2008). Similar to the study presented in Chapter 2, we found that oxytocin reduced activation in the amygdala during exposure to laughter. Reduced amygdala activation might reflect the anxiolytic effects of oxytocin and might enable parents to be more open to the rewarding characteristics of infant laughter. In addition, oxytocin increased connectivity between the amygdala and OFC and ACC. Increased functional connectivity between the OFC, ACC, and amygdala may promote mother-infant bonding by enhancing cognitive control over negative emotionality and at the same time increasing the incentive salience of infant laughter (Berridge, 2007; Berridge & Kringelbach, 2008; Bos et al., 2012; Kringelbach, 2005). The findings of this study are in line with a neural model on the effects of neuropeptides on brain connectivity proposed by Bos et al. (2012), in that they confirm that hormones influence social behavior by affecting the connectivity with the amygdala and other brain regions.

The studies in Chapter 2 and 3 indicate that the association between oxytocin and increased parental sensitivity is partly due to the influence of oxytocin on responses to infant crying and laughing. Oxytocin sensitizes neural circuits involved in the processing of infant signals, which promotes correct perception and adequate responding to the infant. The findings of the studies also indicate that individual differences in oxytocin levels might explain why some parents do not perceive their smiling or laughing infant as a reward (e.g. in postpartum depression) and why some parents find it difficult to respond to their crying infant in a sensitive way. Oxytocin lowers anxious and aversive feelings and stimulates warm feelings for the crying infant. It has been demonstrated that empathic emotional reactions motivate sensitive parental behaviors such as soothing or feeding the child, whereas negative emotional reactions have been linked with the use of harsh responses that are aimed at stopping the crying by all means because it is perceived as aversive (Dix, Gershoff, Meunier, & Miller, 2004). Thus, oxytocin may reduce the likelihood of using such harsh caregiving responses.

It is important to note that the participants in these studies were women without children of their own. The samples consisted of women without children in order to reduce hormonal influences associated with differences in parental experience and between men and women, because of the lack of studies investigating oxytocin effects in women (Bos et al., 2012), and because it is especially maternal behavior that affects child developmental outcomes (Cabrera, Fagan, Wight, & Schadler, 2011). It would be interesting to investigate how oxytocin affects neural responses to infant signals in men and parents. Previous studies indicate that sex and parental status can influence neural responses to infant signals. Seifritz et al. (2003) examined brain responses to infant crying and laughing in mothers and fathers and in women and men without children. They found that women but not men showed neural deactivation in the ACC in response to both infant crying and laughing. Moreover, this response pattern changed with parental experience: in the amygdala and other brain regions involved in emotional processing, parents showed stronger activation with crying, whereas nonparents showed stronger activation with laughing. This might indicate that infant crying might be more emotionally salient for parents than for nonparents. In another study, maternal brain responses to own infant faces were compared with unknown infant faces (Strathearn et al., 2009). Dopamine-associated reward processing regions were found to be significantly more activated during exposure to own happy infant faces compared with unknown happy infant faces. Thus, both sex-specific and experience-dependent neural plasticity shape the human brain response to infant signals (Seifritz et al., 2003), possibly because of the altered oxytocinergic system after child birth, lactation, and parent-infant contact (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman, Weller, Zagoory-Sharon, & Levine, 2007).

Our finding that oxytocin induces changes in brain activation is especially interesting because endocrinologists believe that it is not possible for oxytocin to enter the brain. Oxytocin has poor blood-brain barrier (BBB) permeability, which has been considered an impediment for studying its effects on brain and behavior for a long time (Churchland & Winkielman, 2012). However, the studies presented in this thesis indicate that intranasal administration of oxytocin has significant effects at the neural level. Whereas oxytocin effects at the behavioral level might be influenced by experimenter bias or participants' awareness of the administration, it is highly unlikely that oxytocin effects at the neural level can be explained by these factors. Intranasal administration of oxytocin and other neuropeptides might reach the brain/ be effective through a pathway that circumvents the blood-brain barrier (Bos et al., 2012; Churchland & Winkielman, 2012), in contrast to intravenous administration of neuropeptides, which has been shown to be ineffective in affecting neural activity (Pietrowsky, Struben, Molle, Fehm, & Born, 1996). Intranasal oxytocin might be taken up by pathways allowing for direct effects in the brain, for example via olfactory bulb pathways directly to cerebrospinal fluid (CSF) and the brain or trigeminal nerve pathways to the brainstem (Graustella & Macleod, 2012). The suggestion that intranasal administration of neuropeptides allows direct access to the brain is supported by Born et al. (2002)'s results showing that intranasal arginine vasopressin, a

neuropeptide that is closely related to oxytocin, led to an increase of arginine vasopressin in CSF. Nevertheless, it remains unknown how exactly oxytocin enters the brain and whether it reaches the receptor sites in the brain. Further research is needed to investigate whether nasal administration of oxytocin is the optimal approach to influence social behavior and cognition and to compare effects of various oxytocin administration methods. Moreover, future research should take into account factors influencing absorption of intranasal oxytocin, such as anatomy of the nose and airways, nasal cavity environment and bottle design (Graustella & Macleod, 2012).

Another important question is what constitutes a sufficient dose and timing to ensure behavioral and neural effects (Churchland & Winkielman, 2012). Although the majority of studies investigating the effects of intranasal oxytocin on social behavior involve 24 IU oxytocin and testing at 35 to 50 minutes after administration (Van IJzendoorn & Bakermans-Kranenburg, 2012), the studies presented in this thesis indicate that a dose of 16 IU oxytocin is high enough to ensure behavioral and neural effects. Indeed, Van IJzendoorn, Bhandari, Van der Veen, Grewen, and Bakermans-Kranenburg (in press) found that increases in salivary oxytocin levels persist for more than 7 hours after administration of both 16 and 24 IU oxytocin, with no weaker effects of 16 IU oxytocin. The lower dose of 16 IU oxytocin even resulted in slightly higher initial amounts of salivary oxytocin, indicating that for the dose of oxytocin sniffs it is quite possible that "less is more". A feed forward mechanism has been suggested to explain the persistence of intranasal oxytocin effects; treatment with intranasal oxytocin might lead to an increase in the production of endogenous oxytocin (Van IJzendoorn et al., in press).

Adult attachment and responding to infant crying

Individual differences in the parents' mental representation of attachment are known to influence sensitivity to the child's attachment signals and, therefore, the attachment relationship with the parent (Van IJzendoorn, 1995). However, little is known about the neural mechanisms underlying these attachment-related individual differences in parenting behavior. Research has shown that the perception and processing of infant crying might be involved in explaining differences in responding to infants in parents with secure and insecure attachment representations (Leerkes & Siepak, 2006). The study in Chapter 4 of this thesis was designed to shed more light on the perception and processing of infant signals in individuals with different adult attachment representations by examining neural, emotional, and behavioral responses to infant crying. We were specifically interested in amygdala responses to infant crying in secure and insecure individuals as previous studies showed that amygdala hyperreactivity is an indication of hyperemotionality, and is related to depression and anxiety disorders (Rauch et al., 2000; Yang et al., 2010) and to maternal intrusiveness (Atzil, Hendler, & Feldman, 2011). Moreover, the amygdala is involved in the perception of infant signals (see Chapter 2 and 3), in the processing of attachment-related information (Buchheim et al., 2006), and in insecure attachment (Vrtička, Andersson, Grandjean, Sander, & Vuilleumier, 2008). In line with these studies,

we found that individuals with insecure attachment representations showed heightened amygdala activation during exposure to infant crying compared with individuals with a secure representation. In addition, insecure individuals experienced more irritation during the perception of infant crying and used more excessive force as indicated by grip strength using a handgrip dynamometer. These findings are consistent with studies showing that insecure individuals experience more negative emotions during exposure to infant stimuli and tend to process infant cues in a more negative manner (Dykas & Cassidy, 2011; Leerkes & Siepak, 2006). Amygdala hyperactivity might be the mechanism underlying this negative bias and might explain why insecure individuals respond inconsistently to infant signals or reject their infants' attachment behavior.

Contrary to our expectations, the relation between adult attachment and emotional or behavioral responses to crying was not mediated by amygdala hyperreactivity. Thus, feelings of irritation and the use of excessive force in response to infant crying in insecure individuals can not be solely explained by a hyperactive amygdala. The concept of functional connectivity might explain why we did not find evidence for a mediational model. The amygdala is part of a larger emotion circuitry that consists of brain regions that are functionally connected to each other. These functional connectivities play an important role in emotional regulation and, therefore, in the correct perception and adequate responding to infant stimuli (Bos et al., 2012). For example, prefrontal regions such as the ACC and OFC exert strong regulatory influence over the amygdala, which is important for the regulation of negative emotions (Banks, Eddy, Angstadt, Nathan, & Phan, 2007; Dannlowski et al., 2009; Pillay, Gruber, Rogowska, Simpson, & Yurgelun-Todd, 2006). In addition, in the study presented in Chapter 2 we found that not only the amygdala plays a role in parenting, empathy-related brain regions such as the insula and inferior frontal gyrus are also involved in responding to infant crying. Thus, although the amygdala plays an important role in both the perception of crying and insecure attachment, it might not be the only brain region associated with attachment-related differences in responding to crying. Parental responses to infant crying require activation of multiple cortical and subcortical neural systems involved in functions ranging from the processing of visual and auditory information to complex processes such as affection, emotional regulation, and memory. Because the attachment system most likely relies on this comprehensive neural network, it might not be possible to explain insensitive responsiveness in insecure adults by malfunctioning or dysregulation of a single neural region.

If the relation between adult attachment and responding to crying is not mediated by amygdala hyperactivity, what other factors might underlie this relation? One candidate is oxytocin. In a recent study, Pierrebumbert, Torrisi, Ansermet, Borghini, & Halfon (2012) examined oxytocin responses to stress in individuals with different adult attachment representations. Oxytocin levels were measured before, during and after an experimental psychosocial challenge (the Trier Social Stress Test). Although no attachment-related differences in oxytocin increases or decreases were found, the overall levels of oxytocin differed between the attachment classifications; secure adults had significantly higher oxytocin

levels than insecure adults. In addition, Strathearn et al. (2009) investigated oxytocin levels in mothers varying in adult attachment representations. They found that oxytocin responses to infant contact were higher in secure mothers compared to mothers with dismissing attachment representations. Moreover, oxytocin levels after infant contact correlated with neural responses to own infant faces, measured with fMRI. Thus, oxytocin levels might be involved in explaining attachment-related influence on responding to crying. Future studies should investigate the role of oxytocin in the relation between adult attachment and responding to crying.

The role of context and family background in shaping the effects of oxytocin

Many studies have shown that oxytocin stimulates social behavior (Van IJzendoorn & Bakermans-Kranenburg, 2012). Recent research indicates that the effects of oxytocin are more nuanced than previously thought (Bartz et al., 2011). In Chapter 5, 6 and 7 we investigated the role of a contextual factor and individual differences in moderating the effects of oxytocin on social behavior. Chapter 5 focused on the influence of intranasally administered oxytocin on the perception of infant crying at different frequencies in systematically varied contexts. We examined the differential effects of oxytocin on neural responding to crying that was indicated as coming from a sick infant and crying coming from a bored infant. We were specifically interested in amygdala, insula and IFG responses to crying, because these regions were affected by intranasal oxytocin during exposure to crying in our previous study (Chapter 2). We found that intranasally administered oxytocin led to more pronounced differences in neural responding to sick infant crying compared with bored infant crying. Oxytocin significantly increased insula and IFG responding to crying of a sick infant, but decreased activation in these brain regions during exposure to crying of an infant that was labeled as bored. This may indicate that oxytocin increases concern for infants who are sick and decreases empathic responses to bored infants, thereby lowering the perceived urgency of infant fussiness. This pattern of neural activation may contribute to adequate caregiving responses in both contexts: prompt responding is considered to be an adequate response when the infant is sick, whereas a delayed response to a fussy infant is a sensitive response because it enables the infant to learn to cope with situations of mild distress (Hubbard & Van IJzendoorn, 1991). In addition, we found that oxytocin decreased amygdala responding to crying at 500 Hz, but increased amygdala responding to crying at 700 Hz. This is partly in line with the results of the study presented in Chapter 2. Crying at 700 Hz crying or even higher frequencies occurs in infants who are sick or in pain (Soltis, 2004). Increased amygdala responses to 700 Hz crying might reflect increased vigilance to signals indicating that the infant is in danger. Thus, although reduced amygdala activation stimulates sensitive responding to crying under normal circumstances, increased amygdala responses to crying at high frequencies might be more adaptive because it triggers reactions to protect the child.

The findings of this study indicate that oxytocin enhances the salience of the context and of the acoustics of crying, thus facilitating the interpretation of

the infant's crying and the selection of an adequate caregiving response. This is in line with previous studies indicating that oxytocin improves processing of social information and increases attention towards social cues (e.g. Rimmele et al., 2009). Increased salience of social cues might explain why the effects of oxytocin are dependent on context. Social context has a stronger influence on behavior when one is more aware of the contextual social cues. Some studies indicate that oxytocin can even have negative effects on social behavior under specific social circumstances. For example, De Dreu et al. (2010) found that oxytocin enhanced in-group trust but also promoted defensive aggression toward individuals perceived as out-group members. Aggressive responses after oxytocin administration were most likely to occur when out-group threat is high (De Dreu, Greer, Handgraaf, Shalvi, & Van Kleef, 2012).

In addition to contextual factors, individual differences also influence the effects of oxytocin on social behavior (Bakermans-Kranenburg et al., 2012; Bartz et al., 2011; Van IJzendoorn, Huffmeijer, et al., 2011). In Chapter 6, we examined the influence of oxytocin on prosocial helping behavior toward an excluded known person with a sad or neutral facial expression during online ball-toss game called Cyberball (Williams & Jarvis, 2006), taking into account experiences of maternal use of love withdrawal as a moderating factor. Participants were led to believe that they were playing Cyberball with the experimenter and two other unknown female individuals. After a first fair play block in which all participants received one fourth of the ball throws, the experimenter was excluded from the game and did not receive any throws from the two unknown players. Following the exclusion, the facial expression of the excluded experimenter changed to a sad expression or stayed neutral. This study is the first to indicate that individuals compensate for other players' ostracism by throwing the ball more often to an excluded person, thereby facing the risk of being excluded themselves. In addition, we found that oxytocin further increased the number of ball throws toward the excluded person, possibly because of enhanced empathic feelings and understanding of the emotions of the victim and an increased willingness to take social risks. However, the beneficial effects of oxytocin were only brought about in individuals who experienced a supportive rearing environment. Individuals who experienced high levels of maternal love withdrawal did not show increased prosocial helping behavior toward the victim of the exclusion after oxytocin administration.

Chapter 7 presents a study in which we used resting state fMRI to examine the differential effects of oxytocin in individuals with different family backgrounds at the neural level. 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. The results of our study indicate that oxytocin induced functional connectivity changes between the precuneus/posterior cingulate cortex (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.

The PCC is considered the main functional connectivity hub in the resting brain (Tomasi & Volkow, 2011). It is part of the default mode network, an organized network of brain regions whose activity is ongoing during rest and suppressed during performance of externally cued tasks (Raichle et al., 2001). This network has been suggested to be important for self-referential thought (Anticevic et al., 2012) and social cognition (Schilbach, Eickhoff, Rotarska-Jagiela, Fink, & Vogeley, 2008). Dysregulation of this network has been associated with a number of diseases such as Alzheimer's disease, depression, and schizophrenia (Greicius, 2008). Functional connectivity studies provide more insight into the organization of the PCC and other brain regions within the default mode network. The PCC has strong connections with other brain regions (Buckner et al., 2009; Tomasi & Volkow, 2010), including the thalamus, amygdala, the brainstem, the frontal cortex and the cerebellum (Cavanna & Trimble, 2006; Krienen & Buckner, 2009). PCC connectivities are important for self-referential processes such as self-consciousness, sense of agency, and self-reflection. For example, Alalade, Denny, Potter, Steffens, and Wang (2011) showed that connectivity between the PCC and cerebellum connectivity is altered in patients with depression and suggested that this could represent heightened rumination during resting state. Thus, our finding that oxytocin induced functional connectivity changes with the PCC may indicate that it affects self-referential processes.

Our finding that oxytocin changes PCC-brainstem coupling is in line with previous studies showing that oxytocin affects brainstem connectivity. For example, Kirsch et al. (2005) found that oxytocin reduced coupling of the amygdala to brainstem regions implicated in autonomic and behavioral manifestations of fear during exposure to fearful social stimuli. A reduction of amygdala coupling to regions mediating fear response might be one of the mechanisms underlying the anxiolytic effects of oxytocin. In our study, we did not find significant connectivity changes between the amygdala and brainstem, possibly because of the absence of fearful stimuli.

Interestingly, we found that PCC-cerebellum connectivity changes after oxytocin administration were absent in individuals with a harsh caregiving background. In addition, connectivity changes between the PCC and postcentral gryus, a brain region involved in the experience of pleasant and human touch (Hua et al., 2008; McCabe, Rolls, Bilderbeck, & McGlone, 2008), was also absent in individuals with harsh caregiving experiences. This indicates that individual differences in family background can moderate the effects of oxytocin not only at the behavioral level but also at the neural level. Although the cerebellum has been traditionally associated with motor function, recent studies indicate that it also plays a role in cognition and emotion (Schmahmann, 2010; Stoodley, 2011). The cerebellum is known to be more influenced by environmental factors during development than other brain regions (Giedd, Schmitt, & Neale, 2007) and maturation of the cerebellum has been shown to be affected by harsh caregiving experiences (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009). This may partly explain the moderating role of maternal use of love withdrawal for oxytocin effects on connectivity between the PCC and the cerebellum.

What mechanism might underlie the moderating influence of harsh caregiving experiences on the effects of oxytocin? It has been suggested that methylation, an epigenetic mechanism that controls gene expression by affecting messenger RNA transcription, might explain why the beneficial effects of oxytocin are absent in individuals with a harsh caregiving background (Van IJzendoorn, Caspers, Bakermans-Kranenburg, Beach, & Philibert, 2010; Van IJzendoorn, Huffmeijer, et al., 2011). Methylation is one of the most studied epigenetic means of silencing of genes (Tamashiro & Moran, 2010; Van IJzendoorn, Bakermans-Kranenburg, & Ebstein, 2011). It is considered an important mechanism influencing child development (Van IJzendoorn, Bakermans-Kranenburg, et al., 2012). Several studies have shown that methylation might be a biological basis for the impact of child abuse and neglect on developmental outcomes. For example, Beach, Brody, Todorov, Gunter, and Philibert (2011) found that child sexual abuse increased methylation levels at 5HTTLPR. In addition, McGowan et al. (2009)showed that suicide victims with a history of child abuse (compared with suicide victims without experiences of childhood abuse) have decreased glucocorticoid receptor gene expression through methylation, which is known to influence the regulation of the stress response. Methylation might thus be one of the mechanisms in which the environment prepares the individual for the stressful life that is to be expected (Champagne, 2008; Van IJzendoorn, Bakermans-Kranenburg, et al., 2011). A similar mechanism might explain why the effects of intranasal oxytocin are absent in individuals who experienced harsh caregiving. Early adversity might affect methylation levels of genetic regions regulating the oxytocin system, which might lead to lower oxytocin levels and/or a decreased sensitivity to intranasal OT. This is in line with studies showing dysregulations of the oxytocin system after child abuse and neglect (Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005; Heim et al., 2009; Meinlschmidt & Heim, 2007).

Limitations

Several limitations of the studies presented in the current dissertation should be mentioned. First, our findings can only be generalized to women without children. Effects of oxytocin and adult attachment representations might be different in men or in parents because of hormonal differences. Second, we used a between-subject design to study the effects of oxytocin on social behavior. The use of a between-subjects design implies the risk of pre-existing differences between the oxytocin and placebo group that might have influenced the results. However, randomization and double-blind application have decreased this risk substantially. Moreover, the studies presented in Chapters 2, 3, and 7 were based on samples of monozygotic and dizygotic twins, perfectly matched on age and global child-rearing experiences and even on genotype in monozygotoc twin pairs. Another limitation is that we used self-report questionnaires to study the influence of harsh caregiving experiences on the effects of intranasal oxytocin. In addition, a limitation of the study about the influence of adult attachment on responding to crying (Chapter 4) is the small sample size, which led to the combination of insecure classifications in the analyses and to relatively low power for the analyses. The combination of time-consuming AAI research and

an expensive fMRI investigation is an almost impossible mission for a single research group. Future research should examine the neurobiological processes underlying the separate dismissing, preoccupied, and unresolved attachment representations in larger samples, for example by collaboration of several research teams. Lastly, in this thesis we focused on the effects of oxytocin on social behavior, without examining influences of other hormones. Oxytocin acts in concert with other hormones such as testosterone, cortisol and arginine vasopressin (Bos et al., 2012). Many studies indicate that these hormonal systems are interrelated and that increasing levels of one hormone will induce changes in other hormonal systems. For example, Meinlschmidt and Heim (2007) showed that intranasal administration of oxytocin leads to decreased cortisol levels in healthy participants, but not in individuals who had experienced early parental separation. Thus, future studies should examine the effects of oxytocin in the context of other hormone systems.

Clinical implications and future directions

The studies presented in the current dissertation indicate that oxytocin plays an important role in sensitive parenting and that intranasal administration of oxytocin enhances the processing of infant signals, thereby facilitating sensitive caregiving responses. Given the anxiolytic and prosocial effects of oxytocin, attention for a potential role for intranasal oxytocin administration in the treatment of mental health problems is increasing. Treatment with intranasal oxytocin has been studied in a range of psychiatric disorders, including autism (Bartz & Hollander, 2008), social anxiety disorders (Labuschagne et al., 2010), post traumatic stress disorder (Pitman, Orr, & Lasko, 1993), depression (Pincus et al., 2010), schizophrenia (Feifel, 2012), and borderline personality disorder (Simeon et al., 2011). Support for the idea that intranasal oxytocin is effective in clinical settings comes from a study that showed that elevated salivary oxytocin levels persist for more than seven hours after intranasal administration (Van IJzendoorn et al., in press). Intranasal oxytocin might also be a potential treatment for troublesome parent-child relationships. In a recent study, Mah, Van IJzendoorn, Smith, & Bakermans-Kranenburg (2013) examined the effects of intranasal oxytocin on parenting related expressed emotion in mothers with a postpartum depression. They found that oxytocin did not make depressed mothers happier but their perception of the relationship with their baby improved. The authors suggest that treatment with intranasal oxytocin might show some unwanted side-effects in depressed individuals. Thus, although oxytocin may be a promising potential therapeutic intervention targeting the treatment of several psychiatric disorders and the improvement of insensitive parenting, more research to explore the use of oxytocin in clinical practice is needed.

Our finding that the effects of intranasal oxytocin are dependent on caregiving experiences indicates that there are limitations to the use of intranasal oxytocin as a pharmacotherapy. Oxytocin has positive effects in individuals with supportive family backgrounds, whereas individuals who are most in need of such an intervention because of their harsh caregiving experiences do not profit from the beneficial effects of oxytocin on social behavior. Moreover, oxytocin seems to be

less effective in psychiatric disorders that are caused by etiological factors rooted in negative childhood experiences (Bakermans-Kranenburg & Van IJzendoorn, submitted for publication). Thus, we should be cautious in treating oxytocin as a wonder drug that cures all psychiatric disorders and maladaptive parenting behaviors. Although intranasal oxytocin might be ineffective as a stand-alone intervention to enhance parental sensitivity, a useful strategy to improve troubled parent-child relationships might be to combine intranasal oxytocin with a psychosocial intervention (Bakermans-Kranenburg et al., 2012; Van IJzendoorn & Bakermans-Kranenburg, 2012), such as the Video-feedback Intervention to promote Positive Parenting (VIPP) (Juffer, Bakermans-Kranenburg, & Van IJzendoorn, 2008). Intranasal oxytocin may lead to enhanced perception and processing of the emotions of the child, thereby contributing to the effectiveness of interventions such as the VIPP. In addition, the effects of intranasally administered oxytocin might be different from the effects of endogenous oxytocin secretion. Endogenous oxytocin is released during breastfeeding and after infant affectionate contact (Feldman et al., 2010) and has been shown to have stress-reducing effects in mothers (Heinrichs et al., 2003; Heinrichs et al., 2001). Future research should investigate whether sensitive caregiving can be facilitated in parents by stimulating endogenous oxytocin release, for example by baby massage or breastfeeding. In an ongoing study at the Centre for Child and Family Studies, Leiden University, we are examining the effects of massage at salivary oxytocin levels with a massage chair. Baby massage might be an easy and effective way to enhance oxytocin levels in parents and infants.

Conclusion

The general aim of the current thesis was to gain more insight into the associations between oxytocin, adult attachment and parenting behaviors, and into the role of context and family background in shaping oxytocin effects. We investigated the effects of intranasal oxytocin administration on brain activation and connectivity with a series of functional magnetic resonance imaging studies. Whereas previous studies pointed to a role of oxytocin in parenting, the current dissertation sheds more light on the mechanism underlying the positive association between oxytocin and parenting. Our findings indicate that oxytocin sensitizes caregivers to infant signals by modulating neural circuits related to the perception of infant signals, thereby facilitating sensitive caregiving responses. Thus, individual differences in oxytocin levels may explain why some parents have more difficulty responding to infants in a sensitive way. In addition, the current dissertation sheds more light on the mechanism underlying the negative perception of infant signals in individuals with insecure attachment representations. We found that amygdala hyperactivity might be the mechanism underlying this negative bias and might explain why insecure individuals respond inconsistently to infant signals or reject their infants' attachment behavior. Future research should investigate whether adult attachment and oxytocin levels are two independent factors influencing parenting behaviors or whether they are interrelated.

Furthermore, the current dissertation indicates that the effects of oxytocin are dependent on contextual factors and individual differences. We found that

the prosocial effects of oxytocin were only brought about in individuals who experienced a supportive rearing environment. Individuals who are most in need of an intervention because of their own harsh caregiving experiences do not show increased prosocial behavior after oxytocin administration. Thus, the positive effects of oxytocin on social behavior are moderated by early caregiving experiences, possibly because early social adversity affects methylation levels in genetic areas regulating the oxytocin system, which in turn leads to a decreased sensitivity to intranasal oxytocin. This finding indicates that intranasal oxytocin may be ineffective as a stand-alone intervention to enhance parental sensitivity. Future studies should investigate whether a combination of intranasal oxytocin and a psychosocial intervention, and / or the stimulation of endogenous oxytocin release through e.g. massage, are effective therapies for troubled parent-child relationships.

Nederlandse samenvatting

Als baby's huilen is dat een belangrijk signaal voor ouders. Huilen betekent dat er honger, dorst, of ander ongemak is en roept ouders op tot troosten, voeden en koesteren. Lachen versterkt de band tussen ouder en kind en verhoogt het plezier in de interactie. Omdat baby's volledig afhankelijk zijn van hun ouders is een correcte waarneming en interpretatie van huilen en lachen erg belangrijk voor het overleven van baby's. Het is daarom niet vreemd dat specifieke neurale netwerken en neuroendocriene processen voor de waarneming van babysignalen zich hebben ontwikkeld in het menselijk brein (Newman, 2007). Helaas zijn niet alle ouders altijd in staat om op een sensitieve manier te reageren op de signalen van hun baby. Huilende baby's kunnen irritant zijn en ook lachen wordt niet door alle ouders als een beloning ervaren. Soms kan excessief huilen zelfs leiden tot een hardhandige aanpak. Bijna 6% van de ouders heeft het eigen kind 6 maanden na de geboorte al eens stevig geschud of geslagen in de hoop dat het huilen zou ophouden (Reijneveld, Van der Wal, Brugman, Sing, & Verloove-Vanhorick, 2004). Een belangrijke vraag is daarom welke mechanismen ten grondslag liggen aan het waarnemen van en reageren op babysignalen en welke factoren de individuele verschillen in ouderlijke sensitiviteit kunnen verklaren. Twee belangrijke factoren die ouderlijk gedrag beïnvloeden zijn het hormoon oxytocine en de mentale representatie van gehechtheid van volwassenen. In de studies beschreven in dit proefschrift wordt de invloed van deze factoren op het waarnemen van en reageren op babysignalen onderzocht met functional magnetic resonance imaging (fMRI).

Oxytocine

Oxytocine is een neuropeptide die vooral geproduceerd wordt in de supraoptische en paraventriculaire kernen van de hypothalamus en in het bloed terecht komt via de hypofyse. Dieronderzoek heeft laten zien dat oxytocine een cruciale rol speelt bij de geboorte, lactatie en het ontstaan van de band tussen moeder en kind (Carter, 1998). Ook bij mensen speelt oxytocine een rol in de band tussen ouder en kind en bij andere vormen van sociaal gedrag. Zo blijkt uit studies dat mensen met hoge oxytocine waarden in het bloed meer vertrouwen hebben in anderen (Zak, Kurzban, & Matzner, 2005), vrijgeviger zijn (Barraza & Zak, 2009) en sensitievere ouders zijn dan mensen met lage oxytocine waarden (Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Insel, 2010). Daarnaast hebben verschillende studies laten zien dat een neusspray met oxytocine sociaal gedrag kan stimuleren. Intranasale toediening van oxytocine verhoogt bijvoorbeeld vertrouwen (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005), empathie (Bartz et al., 2010), begrip van emoties (Domes, Heinrichs, Michel, Berger, & Herpertz, 2007) en coöperatief gedrag (Rilling et al., 2012). Deze studies laten zien dat er

een causaal verband is tussen oxytocine en sociaal gedrag, in tegenstelling tot correlationele studies waarbij causaliteit niet kan worden bewezen. De causale rol van oxytocine in ouderlijk gedrag is echter nog maar weinig onderzocht. Het is bovendien onduidelijk *hoe* oxytocine ouderlijk gedrag beïnvloedt.

In de studies beschreven in Hoofdstuk 2 en 3 van dit proefschrift wordt de rol van oxytocine in ouderlijk gedrag belicht. In deze studies werd het effect van oxytocine op neurale reacties op babysignalen onderzocht met fMRI in een steekproef van 42 eeneiige en twee-eiige tweelingzussen. De tweelingparen werden opgesplitst zodat de ene tweelingzus een neusspray met oxytocine kreeg en de andere een neusspray met een placebo-oplossing. Deze 'tussen-proefpersonen' onderzoeksopzet met tweelingen heeft als voordeel dat de oxytocine en placebo groep niet of nauwelijks verschilden op kenmerken die invloed kunnen hebben op de gevoeligheid voor een oxytocine neusspray en op reacties op huilende en lachende baby's, zoals opvoeding, leeftijd en zelfs (voor eeneiige tweelingparen) genotype.

Na de toediening van de neusspray kregen de participanten een reeks huil- en lachgeluiden van baby's te horen in de MRI scanner. Hun hersenactiviteit werd gemeten en vergeleken met hun hersenactiviteit tijdens controlegeluiden met dezelfde akoestische kenmerken maar waarin geen huilen of lachen te herkennen was. Uit deze studies bleek dat oxytocine de activiteit in de amygdala verlaagde tijdens het luisteren naar babygehuil in verglijking met controle geluiden. De amygdala is een hersengebied dat deel uit maakt van het limbische systeem en een rol speelt bij angst en afkeer (LeDoux, 2000). Oxytocine bevordert dus mogelijk sensitief reageren op huilen door angst en afkeer te remmen. Daarnaast verhoogde oxytocine de activiteit in de insula en de inferieure frontale gyrus, hersengebieden die belangrijk zijn voor empathie en begrip van emoties (Chakrabarti, Bullmore, & Baron-Cohen, 2006; Decety & Jackson, 2004; Jabbi, Swart, & Keysers, 2007). De effecten van oxytocine zijn dus mogelijk tweeledig: oxytocine bevordert sensitief reageren op huilen door te voorkomen dat ouders overweldigd worden door negatieve emoties, en door empathische gevoelens voor de baby te stimuleren.

We vonden dat oxytocine ook tijdens het luisteren naar lachende baby's de activiteit in de amygdala remde. Daarnaast versterkte oxytocine de functionele connectiviteit, ofwel de correlatie in activiteit tussen hersengebieden, in dit geval tussen de amygdala en twee beloningsgebieden in de hersenen, te weten de orbitofrontale cortex en de anterieure cingulate cortex. Een lage activiteit in de amygdala in combinatie met sterkere connectiviteit met neurale beloningsgebieden kan ervoor zorgen dat ouders meer open staan voor de positieve kenmerken van het lachen van baby's. Onze resultaten wijzen er dus op dat oxytocine het luisteren naar een lachende baby aantrekkelijker maakt.

De studies beschreven in Hoofdstuk 2 en 3 geven aan dat oxytocine ouderlijke sensitiviteit stimuleert door de waarneming van huilen en lachen van baby's te beïnvloeden. Oxytocine verbetert de informatieverwerking van babysignalen en dit kan bijdragen aan een correcte waarneming en een adequate reactie op het babysignaal. De bevindingen van deze studies wijzen erop dat individuele verschillen in oxytocine niveaus mogelijk verklaren waarom sommige ouders

hun lachende baby niet als een beloning ervaren (bijvoorbeeld bij postnatale depressie) en waarom sommige ouders het moeilijk vinden om op een sensitieve manier te reageren op hun huilende baby. Het is echter belangrijk om op te merken dat de proefpersonen in de studies beschreven in dit proefschrift vrouwen zonder kinderen waren. We kozen voor vrouwen zonder kinderen om hormonale invloeden gerelateerd aan ouderschap en sekse te voorkomen en omdat vooral moederlijk gedrag invloed heeft op de ontwikkeling van kinderen (Cabrera, Fagan, Wight, & Schadler 2011). Interessant voor vervolgonderzoek is de vraag of oxytocine de waarneming van babysignalen op eenzelfde manier beïnvloedt in mannen en moeders.

Gehechtheidsrepresentaties van volwassenen

De gehechtheidsrepresentatie is een andere belangrijke factor die ouderlijk gedrag beïnvloedt. De gehechtheidsrepresentatie is de cognitieve representatie van gehechtheidservaringen in het verleden en het heden. Deze representatie kan worden bepaald met het Gehechtheidsbiografisch Interview (GBI), een interview van ongeveer een uur waarin wordt gevraagd naar gehechtheidservaringen in de kindertijd en naar de invloed van deze ervaringen op de ontwikkeling en op de persoonlijkheid (Hesse, 2008; Main & Goldwyn, 1984). De transcripten van het GBI worden gecodeerd als veilig, onveilig-gereserveerd of onveilig-gepreoccupeerd. Personen die als veilig worden geclassificeerd vinden gehechtheidsrelaties belangrijk en beschrijven gehechtheidservaringen op een coherente manier. Personen met een onveilig-gereserveerde gehechtheidsrepresentatie hebben meer moeite de relatie met hun ouders op een coherente manier te beschrijven. Ze idealiseren hun ouders of andere gehechtheidsfiguren terwijl ze geen goede voorbeelden kunnen geven voor de positieve kenmerken van de relatie met hun ouders. Daarnaast worden gehechtheidservaringen door deze personen als onbelangrijk bestempeld en niet van invloed op de ontwikkeling. Ook personen met een onveilig-gepreoccupeerde representatie hebben moeite de relatie met hun ouders op een coherentie manier te beschrijven. Transcripten van deze personen worden gekenmerkt door lange antwoorden en boosheid over het gedrag van hun ouders. Verder benadrukken personen met een gepreoccupeerde representatie de negatieve invloed van gehechtheidservaringen op hun ontwikkeling.

Uit onderzoek blijkt dat de gehechtheidsrepresentatie van de ouder invloed heeft op ouderlijke sensitiviteit (Van IJzendoorn, 1995). Ouders met onveilige gehechtheidsrepresentaties reageren minder sensitief op signalen van hun kinderen dan ouders met een veilige gehechtheidsrepresentatie. Ze reageren inconsistent of wijzen de gehechtheidssignalen van hun kind af. Onveilige ouders zijn ook minder goed in staat om emoties van kinderen correct te identificeren en denken vaker dat een kind huilt om negatieve redenen (bijvoorbeeld omdat het kind verwend is of een moeilijk karakter heeft) (Leerkes & Siepak, 2006). Onveilige ouders hebben dus de neiging om signalen van baby's op een negatieve manier waar te nemen en dit kan bijdragen aan insensitief ouderschap en onveilige gehechtheid van het kind (Dykas & Cassidy, 2011). In Hoofdstuk 4 is de invloed van gehechtheidsrepresentatie op de waarneming van huilen van baby's onderzocht met fMRI in een steekproef van 21 vrouwen zonder kinderen.

Personen met een onveilige gehechtheidsrepresentatie bleken meer activiteit in de amygdala te laten zien tijdens het luisteren naar huilen in vergelijking met controle geluiden. Dit geeft mogelijk aan dat personen met een onveilige gehechtheidsrepresentatie meer negatieve gevoelens ervaren tijdens het luisteren naar huilen en verklaart wellicht waarom ouders met deze representatie meer moeite hebben om op een sensitieve manier te reageren op hun huilende baby.

Ook onderzochten we emotionele reacties op huilen en hoe hard participanten knijpen als reactie op huilen, gemeten met een handgreep dynamometer. Eerder onderzoek heeft aangetoond dat te hard knijpen tijdens luisteren naar babygehuil gerelateerd is aan risico op kindermishandeling en sociaal dominant gedrag (Crouch, Skowronski, Milner, & Harris, 2008; Gallup, O'Brien, White, & Wilson, 2010) en dat het aantal keer dat te hard wordt geknepen door personen met positieve opvoedingservaringen afneemt na oxytocine toediening (Bakermans-Kranenburg, Van IJzendoorn, Riem, Tops, & Alink, 2012). In de studie beschreven in Hoofdstuk 4 werd aan de participanten gevraagd met maximale kracht in de dynamometer te knijpen en daarna op halve kracht. Het maximaal en halve kracht knijpen werd geoefend totdat de participanten in staat waren om tijdens de tweede knijppoging inderdaad half keer zo hard te knijpen als tijdens de eerste knijppoging. Vervolgens werden de participanten geïnstrueerd om vier keer maximaal en op halve kracht te knijpen tijdens het luisteren naar lachende en huilende baby's.

We vonden dat participanten met een onveilige gehechtheidsrepresentatie vaker te hard knepen tijdens het luisteren naar huilen. Bovendien rapporteerden deze participanten meer irritatie vergeleken met participanten met een veilige gehechtheidsrepresentatie. Dit sluit aan bij eerder onderzoek naar personen met een onveilige representatie die babysignalen op een negatieve manier waarnemen en verwerken (Leerkes & Siepak, 2006). Ook onderzochten we of de relatie tussen onveilige gehechtheidsrepresentatie enerzijds, en meer irritatie en harder knijpen anderzijds kon worden verklaard door overmatige amygdala activiteit. In tegenstelling tot onze verwachting was dat niet het geval. Negatieve gevoelens en te hard knijpen bij personen met een onveilige gehechtheidsrepresentatie kunnen dus niet alleen worden verklaard door een overactieve amygdala. Mogelijk speelt de connectiviteit tussen de amygdala en andere hersengebieden die belangrijk zijn voor emotionele processes (bijvoorbeeld de insula, inferieure frontale gyrus, orbitofrontale cortex en de anterieure cingulate cortex) ook een rol in de reacties op huilen bij personen met een onveilige gehechtheidsrepresentatie.

Oxytocine: de invloed van context en individuele verschillen

De afgelopen tien jaar is er veel onderzoek gedaan naar de gunstige effecten van oxytocine op sociaal gedrag (voor reviews zie Graustella & Macleod, 2012; Van IJzendoorn & Bakermans-Kranenburg, 2012). Recente studies tonen echter aan dat de effecten van oxytocine complexer zijn dan eerder gedacht (Bartz, Zaki, Bolger, & Ochsner, 2011; Bakermans-Kranenburg et al., 2012). Contextuele factoren en individuele verschillen lijken de effecten van oxytocine op sociaal gedrag te modereren. Oxytocine verhoogt bijvoorbeeld alleen het vertrouwen in anderen als die ander bekend is of als een betrouwbaar persoon wordt beschouwd (Declerck,

Boone, & Kiyonari, 2010; Mikolajczak et al., 2010). De Dreu (2010) onderzocht met zijn onderzoeksgroep de effecten van oxytocine in verschillende sociale situaties en vond dat oxytocine in sommige situaties zelfs negatieve effecten kan hebben. Zo verhoogde oxytocine altruïsme voor mensen die beschouwd werden als lid van eenzelfde groep, maar tegelijkertijd ook agressieve reacties naar mensen die beschouwd werden als een buitenstaander. De sociale context heeft dus invloed op de effecten van oxytocine op sociaal gedrag. Het is echter nog onbekend *of* en *hoe* sociale context en individuele verschillen de effecten van oxytocine op ouderlijk gedrag beïnvloedt.

In Hoofdstuk 5 werd onderzocht wat de invloed is van sociale context op de effecten van intranasale oxytocine op neurale reacties op gehuil van baby's in een steekproef van 54 vrouwen zonder kinderen. De participanten luisterden in de MRI scanner naar babyhuilen waarbij werd aangegeven dat het van een zieke baby afkomstig was of van een baby die zich verveelde. We vonden dat de verschillen tussen neurale reacties op huilen dat werd getypeerd als afkomstig van een zieke baby in vergelijking tot datzelfde huilen getypeerd als afkomstig van een verveelde baby groter waren na oxytocine toediening in vergelijking met toediening van een placebo neusspray. Oxytocine verhoogde de activiteit in de insula en inferieure frontale gyrus tijdens het luisteren naar huilen van een zieke baby, maar verlaagde de activiteit in deze hersengebieden tijdens het luisteren naar verveeld babyhuilen. Dit kan in beide situaties bijdragen aan een adequate ouderlijke reactie op huilen. Een snelle ouderlijke reactie is namelijk belangrijk als baby's huilen omdat ze ziek zijn, terwijl een vertraagde ouderlijke reactie op verveeld gehuil adequaat is omdat baby's dan kunnen leren om te gaan met situaties van lichte stress (Hubbard & Van IJzendoorn, 1991).

Daarnaast vonden we dat oxytocine de verschillen tussen neurale reacties op huilen op lage en hoge toonhoogtes vergrootte. Oxytocine bleek de activiteit in de amygdala te verlagen tijdens luisteren naar huilen op een frequentie van 500 Hz, terwijl de activiteit in de amygdala hoger werd tijdens het luisteren naar huilen op een frequentie van 700 Hz. Verhoogde amygdala activiteit tijdens hoge huilgeluiden kan er op wijzen dat mensen meer alert zijn op signalen die aangeven dat het kind in gevaar is. Oxytocine verhoogt dus niet alleen de aandacht voor de contextuele informatie maar ook voor akoestische kenmerken van babyhuilen. Beide zijn belangrijke informatiebronnen voor ouders omdat ze informatie geven over de gezondheid van het kind en de mate van stress die het kind ervaart. Zo huilen baby's die ziek zijn of pijn hebben op hogere toonhoogtes (Soltis, 2004). Oxytocine verhoogt dus aandacht voor de akoestische en contextuele informatie over het huilen en dit draagt bij aan een correcte interpretatie van het baby signaal en stimuleert een adequate ouderlijke reactie op huilen.

Naast contextuele factoren beïnvloeden individuele verschillen ook de effecten van oxytocine op sociaal gedrag (Bartz, Zaki, Bolger, & Ochsner, 2011). In een eerdere studie vonden we bijvoorbeeld dat intranasale oxytocine het gebruik van te veel handgreep kracht tijdens het luisteren naar babygehuil verminderde, maar alleen bij personen die een sensitieve opvoeding hadden meegemaakt (Bakermans-Kranenburg et al., 2012). De effecten van oxytocine waren afwezig voor personen die hardhandig opgevoed waren door hun ouders. Dit geeft aan

dat niet alleen sociale context maar ook een hardhandige opvoeding de gunstige effecten van oxytocine op sociaal gedrag modereert. In Hoofdstuk 6 hebben we de modererende invloed van hardhandige opvoeding op de effecten van oxytocine verder onderzocht met Cyberball. Cyberball is een virtueel computerspel waarbij de proefpersoon een bal overgooit naar andere personen en waarbij gedurende het spel iemand buitengesloten wordt (Williams & Jarvis, 2006). Het spel is in een aantal studies gebruikt om de effecten van sociale exclusie te onderzoeken (Eisenberger & Lieberman, 2004; Eisenberger, Lieberman, & Williams, 2003; Gonsalkorale & Williams, 2007; Zadro, Williams, & Richardson, 2004). Er zijn echter nog maar weinig studies waarin Cyberball is toegepast om te onderzoeken hoe personen reageren als ze zien dat iemand anders wordt buitengesloten.

In het onderzoek beschreven in Hoofdstuk 6 werd aan de participanten verteld dat ze Cyberball gingen spelen met de proefleider van het onderzoek en twee andere onbekende vrouwelijke spelers. Na een eerste spelronde waarbij alle participanten één vierde van de worpen kregen toebedeeld, kreeg de proefleider geen bal meer toegespeeld van de twee onbekende spelers. Uit de analyses bleek dat participanten de sociale exclusie compenseerden door de bal vaker naar de proefleider te gooien. Daarnaast vonden we dat intranasale oxytocine het aantal worpen naar de buitengesloten proefleider verder deed toenemen. Oxytocine bevordert dus hulp aan een slachtoffer van sociale exclusie, mogelijk omdat oxytocine ervoor zorgt dat mensen eerder bereid zijn om sociale risico's te nemen of omdat oxytocine inleving in de emoties van het slachtoffer verbetert. De gunstige effecten van oxytocine waren echter alleen aanwezig in participanten die een sensitieve opvoeding hadden meegemaakt. Participanten die rapporteerden dat hun moeder de opvoedingsstrategie *love withdrawal* (liefdesonthouding) had gebruikt, gooiden de bal niet vaker naar de buitengesloten persoon na toediening van oxytocine. Liefdesonthouding is een opvoedingsstrategie waarbij ouders een kind liefde onthouden wanneer het kind faalt of iets doet wat niet mag. Overmatig gebruik van deze opvoedingsstrategie wordt gezien als een vorm van psychologische mishandeling (Euser, Van IJzendoorn, Prinzie, & Bakermans-Kranenburg, 2010).

In Hoofdstuk 7 hebben we de invloed van hardhandige opvoeding op de effecten van oxytocine bestudeerd met *resting state fMRI*, een techniek om het brein tijdens rust te bestuderen. Het brein consumeert ongeveer 20% van onze energie tijdens rust, voornamelijk vanwege 'spontane' fluctuaties in neurale activiteit. Deze energieconsumptie tijdens rust is erg hoog vergeleken met de lichte toename in neuraal metabolisme tijdens het uitvoeren van taken (minder dan 5%). Ondanks deze hoge energieconsumptie werden spontane fluctuaties in hersenactiviteit lang gezien als random storingen. Dit beeld veranderde toen studies vonden dat spontane neurale activiteit op een specifieke manier georganiseerd is in het brein in rust (Biswal et al., 2010). Smith en zijn onderzoeksgroep (2009) vonden bijvoorbeeld met een resting state fMRI studie dat de correlaties in spontane activiteit vooral hoog zijn tussen hersengebieden die functioneel gerelateerd zijn aan elkaar. Bovendien hebben verschillende studies laten zien dat functionele connectiviteit tijdens rust afwijkend is bij patienten met psychiatrische stoornissen (Greicius, 2008), zoals dementie (Hafkemeijer,

Van der Grond, & Rombouts, 2011), depressie (Veer et al., 2010) en schizofrenie (Zhou et al., 2007).

Geneesmiddelen kunnen veranderingen in de netwerken van het rustbrein teweeg brengen (Cole et al., in press; Khalili-Mahani et al., 2012; Tanabe et al., 2011). Resting state fMRI kan dus worden ingezet om farmacologische effecten te onderzoeken, zoals effecten van intranasale oxytocine in personen met verschillende opvoedingservaringen. Er is nog maar weinig onderzoek uitgevoerd naar farmacologische effecten op neurale connectiviteit tijdens rust. Bovendien is onbekend of een hardhandige opvoeding de effecten van oxytocine modereert op neuraal niveau. In Hoofdstuk 7 onderzochten we de effecten van intranasale oxytocine op functionele connectiviteit tijdens rust, rekening houdend met 'love withdrawal' als potentiële moderator. Uit de analyses bleek dat oxytocine de functionele connectiviteit met de precuneus/posterieure cingulate cortex (PCC) veranderde. De PCC wordt beschouwd als een van de belangrijkste connectiviteitscentra in het rustbrein. Het is onderdeel van het *default mode network,* een netwerk van hersengebieden die een verhoging in activiteit laten zien tijdens rust (Raichle et al., 2001). De PCC en andere gebieden in het default mode network zijn belangrijk voor denken over jezelf, zelfbewustzijn en sociale cognitie (Anticevic et al., 2012; Schilbach, Eickhoff, Rotarska-Jagiela, Fink, & Vogeley, 2008). Onze bevinding dat oxytocine PCC connectiviteit verandert, geeft dus mogelijk aan dat oxytocine zelfreflectie beïnvloedt.

We vonden veranderingen in connectiviteit tussen de PCC enerzijds en de hersenstam, cerebellum en postcentrale gyrus anderzijds. De postcentrale gyrus is een hersengebied dat betrokken is bij de ervaring van aangename en menselijke aanrakingen (Hua et al., 2008; McCabe, Rolls, Bilderbeck, & McGlone, 2008). Oxytocine zou dus de informatieverwerking van menselijke aanrakingen kunnen bevorderen. Dit sluit aan bij eerder onderzoek dat heeft laten zien dat oxytocineniveaus gerelateerd zijn aan aangenaam fysiek contact tussen ouder en kind (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010). De verandering in PCC-postcentrale gyrus connectiviteit vond echter alleen plaats bij personen die weinig liefdesonthouding hadden ervaren. Participanten die relatief veel liefdesonthouding hadden meegemaakt vertoonden geen veranderingen in connectiviteit tussen de PCC en de postcentrale gyrus. Ook PCC-cerebellum connectiviteit was alleen veranderd na oxytocine toediening bij mensen die een sensitieve opvoeding hadden meegemaakt. Het cerebellum werd lange tijd beschouwd als een hersengebied dat vooral belangrijk is voor motoriek. Recent onderzoek heeft echter aangetoond aan dat het ook een rol speelt in cognitie en emotie (Schmahmann, 2010; Stoodley, 2011). Het cerebellum wordt tijdens de ontwikkeling meer beïnvloed door factoren uit de omgeving dan andere hersengebieden (Giedd, Schmitt, & Neale, 2007). Zo is bekend dat een hardhandige opvoeding invloed heeft op de ontwikkeling van het cerebellum (Bauer, Hanson, Pierson, Davidson, & Pollak, 2009). Dit verklaart mogelijk waarom liefdesonthouding de effecten van oxytocine op de connectiviteit tussen de PCC en cerebellum modereerde, terwijl de effecten van oxytocine op de connectiviteit tussen de PCC en hersenstam niet beïnvloed werden.

Het effect van intranasale oxytocine lijkt dus afhankelijk van sociale context en individuele verschillen (zie ook Bakermans-Kranenburg et al., 2012; Bartz et al., 2011; Van IJzendoorn, Huffmeijer, Alink, Bakermans-Kranenburg, & Tops, 2011). De gunstige effecten van oxytocine op sociaal gedrag en functionele connectiviteit in het rustbrein lijken minder sterk of zelfs afwezig bij personen die geen ondersteunende opvoeding zeggen te hebben meegemaakt. Deze opvoedingservaringen modereren dus de effecten van oxytocine. Mogelijk beïnvloedt een hardhandige of insensitieve opvoeding de werking van het oxytocine systeem, bijvoorbeeld door methylatie in genetische gebieden die het oxytocine systeem reguleren, wat vervolgens zorgt voor een verminderde gevoeligheid voor intranasale oxytocine. Helaas betekent dit dat oxytocine mogelijk minder of niet effectief is bij personen die een interventie wellicht het hardst nodig hebben, namelijk personen die hardhandig of zonder sensitieve ondersteuning zijn opgevoed. Oxytocine lijkt daarom niet geschikt als een op zichzelf staande farmacologische interventie voor de verbetering van insensitief ouderschap. Vervolgonderzoek zou zich kunnen richten op de vraag of een combinatie van oxytocine met een psychologische interventie en/of het stimuleren van oxytocine afgifte (bijvoorbeeld door massage of borstvoeding) effectieve strategieën zijn om problematische ouder-kind relaties te verbeteren.

Conclusie

Het in dit proefschrift beschreven onderzoek geeft inzicht in de relaties tussen oxytocine, gehechtheidsrepresentaties en ouderlijk gedrag. Met gebruik van fMRI zijn de effecten van intranasale oxytocine op neurale activatie en connectiviteit tijdens rust en tijdens luisteren naar babysignalen in kaart gebracht. De resultaten geven aan dat oxytocine sensitief ouderschap stimuleert door de informatieverwerking van babysignalen te verbeteren. Oxytocine bevordert adequate reacties op babysignalen door te voorkomen dat ouders overweldigd worden door negatieve emoties, en door empathische en warme gevoelens voor de baby te stimuleren. Individuele verschillen in oxytocine niveaus kunnen dus verklaren waarom sommige ouders het moeilijk vinden om op een sensitieve manier te reageren op hun huilende baby. Daarnaast laten de resultaten van de studies zien dat de effecten van intranasale toediening van oxytocine afhankelijk zijn van sociale context en individuele verschillen. Oxytocine stimuleert empathische reacties voor zieke baby's en verlaagt tegelijkertijd de actiebereidheid voor baby's die huilen omdat ze zich vervelen. Dit kan in beide situaties bijdragen aan een adequate ouderlijke reactie op huilen. De gunstige effecten van oxytocine vinden echter alleen plaats bij mensen die een sensitieve opvoeding hebben meegemaakt.

Verder bieden de studies beschreven in dit proefschrift meer inzicht in het mechanisme dat ten grondslag ligt aan de negatieve perceptie van babysignalen bij personen met een onveilige gehechtheidsrepresentatie. Overactiviteit van de amygdala verklaart mogelijk waarom ouders met een onveilige gehechtheidsrepresentatie meer moeite hebben om op een sensitieve manier te reageren op hun baby. Het is daarbij de vraag of oxytocine en de gehechtheidsrepresentatie twee onafhankelijke factoren zijn die ouderschap beïnvloeden of dat ze met elkaar in verband staan en elkaar versterken.

Dankwoord

Dit proefschrift was niet tot stand gekomen zonder de hulp, steun en inspanningen van vele anderen. Ik wil iedereen bedanken die hieraan heeft bijgedragen. Ten eerste wil ik de proefpersonen bedanken voor hun deelname aan de studies. Ook hartelijk dank aan de studenten die mee hebben geholpen met de verzameling van de fMRI data. Daarnaast wil ik mijn collega's van Algemene- en Gezinspedagogiek bedanken voor voor de prettige samenwerking. In het bijzonder wil ik Dorothée en Suzanne bedanken voor de hulp tijdens de eerste periode van mijn promotietraject. Claudia, Sandra, Rianne, Mariëlle en alle andere aio's: bedankt voor de ontspanning en afleiding. Lieve familie en vrienden, bedankt voor jullie steun, interesse, humor en belangstelling. Pap, mam en Daniëlle, heel erg bedankt voor de steun en het vertrouwen dat jullie altijd in mij hebben gehad. Last but not least, lieve Tom: bedankt voor alles!

Curriculum Vitae

Madelon Hendricx-Riem werd geboren op 22 september 1984 in Maastricht. In 2002 haalde zij haar VWO diploma aan het Trichter College in Maastricht. Aansluitend begon zij aan de opleiding Psychologie aan de Radboud Universiteit in Nijmegen waar zij koos voor de specialisatie Neuro- en Revalidatiepsychologie. Na het behalen van haar bachelor begon zij aan de twee-jarige researchmaster Cognitive Neuroscience die zij in 2008 met genoegen afrondde en de master Neuro- en Revalidatiepsychologie die zij in 2009 cum laude afrondde. Zij deed klinische ervaring op tijdens een stage op de afdeling klinische psychologie van het Canisius Wilhelmina Ziekenhuis in Nijmegen en schreef haar scriptie over spiegelneuronen en autisme. Tijdens haar studie deed zij onderzoekservaring op als student-assistent bij het Baby Research Center, Radboud Universiteit, Nijmegen. Sinds haar afstuderen is zij werkzaam bij de afdeling Algemene- en Gezinspedagogiek (AGP), Universiteit Leiden. Haar promotieonderzoek was gericht op de invloed van oxytocine en gehechtheidsrepresentaties op neurale reacties op babysignalen. De resultaten van haar onderzoek zijn beschreven in dit proefschrift. Naast haar aanstelling als promovenda is Madelon ook één dag in de week aangesteld als docent bij AGP. Daarnaast heeft zij ervaring opgedaan als psycholoog bij De Praktijk Leiderdorp.

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Oxytocin receptor gene and depressive symptoms associated with physiological reactivity to infant crying

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Abstract

Both the oxytocin receptor (OXTR) gene and depressive symptoms have been associated with parenting behavior. The OXTR GG genotype has been suggested to be related to more sensitive parenting, whereas depressive symptoms may affect sensitivity negatively. We examined the role of OXTR and the influence of depressive symptoms in explaining differences in physiological reactivity to infant crying. Heart rate responses of 40 healthy females without children (age 19-47 years, randomly selected half of twin pairs) were measured during the presentation of three episodes of infant cry sounds. Participants with the presumably more efficient variant of the oxytonergic system gene (OXTR GG) had more pronounced physiological reactivity to repeated cry sounds, except when they showed more symptoms of depression. Results were replicated in the second half of the twin sample. This is the first study to suggest effects of OXTR genotype on physiological reactivity to infant crying. Depressive symptoms may however suppress the effect of the OXTR GG genotype.

Introduction

The infant cry is a signal of distress evolved to elicit parental proximity and caregiving (Bowlby, 1969/1982, Zeifman, 2001). Infant crying evokes physiological reactions in adults that are associated with prompt caregiving responses (Del Vecchio, Walter, & O'Leary 2009). Genetic factors have been shown to contribute to individual differences in physiological reactivity to infant crying (Out, Pieper, Bakermans-Kranenburg, & Van IJzendoorn, 2010). More specifically, the oxytocin receptor (OXTR) may be involved in explaining the variance in maternal sensitivity (Bakermans-Kranenburg and Van IJzendoorn, 2008; Feldman et al., 2007), defined as the ability to accurately perceive children's signals and to respond in an adequate and prompt way (Ainsworth, Blehar, Waters, & Wall, 1978). Maternal sensitivity might be associated with more pronounced physiological reactivity to infant crying, which in turn may be related to OXTR genotype. At the same time, depressive symptoms are expected to decrease physiological reactivity to infant crying, since depressed mothers have more often been found to be less responsive to their infant's signals than non-depressed mothers (Donovan, Leavitt, & Walsh, 1998; Murray and Cooper, 1997; Murray, Fiori-Cowley, Hooper, & Cooper, 1996; Schuetze and Zeskind, 2001). In this study we examined the role of the OXTR gene and depressive symptoms in explaining differences in physiological reactivity to infant crying.

Infant crying provides information about the infant's health and the intensity of distress through the acoustics of the cry sound (Gustafson, Wood, & Green, 2000; Murray, 1979). Cries produce autonomic arousal in adults, such as elevated heart rate (HR) and skin conductance (Crowe and Zeskind, 1992; Frodi, Lamb, Leavitt, & Donovan, 1978; Frodi, 1985; Frodi and Lamb, 1980, Wiesenfeld, Malatesta, & Deloach, 1981), and this state of arousal leads to a quick response to the infant in order to terminate the cry (Del Vecchio et al., 2009). In a behavioral genetic study Out et al. (2010) showed that adults' cardiac activity increased during repeated infant distress signals; they reasoned that adults become increasingly sensitized to these signals. In addition, variance in adults' cardiac reactivity to cry sounds was shown to be explained by genetic factors, making some adults physiologically more reactive to cry stimuli than others. Bakermans-Kranenburg and Van IJzendoorn (2008) found higher levels of sensitive responsiveness to toddlers in parents with the OXTR rs53576 GG genotype, the potentially more effective variant of the oxytonergic system gene, than in parents with OXTR AA and AG genotypes. The link between OXTR gene and parenting has been suggested as a promising direction for future research into parenting (Taylor, 2008). The role of oxytocin in parenting has been demonstrated in several animal studies (see for a review Carter, 1998). A recent study involving human mothers showed that oxytocin levels across pregnancy and the postpartum period were positively related to sensitive parenting in the first month after birth (Feldman, Weller, Zagoory-Sharon, & Levine, 2007). Oxytocin might play a role in the synchrony of interaction, defined as the matching of behavior, affective states and biological rhythms between parent and child (Feldman, 2007).

Whereas the OXTR GG genotype may increase maternal sensitivity to infants' distress signals, depressive symptoms have been associated with poor mother-infant interaction. Depressed mothers have difficulty providing appropriate social responses during interactions with their infant (Murray et al., 1996) and exhibit fewer affectionate contact behaviors towards their infants (Feldman and Eidelman, 2003; Feldman and Eidelman, 2004). They are less able to track their infant's activities and physically protect them from potential hazards (Gelfand and Teti, 1990). Murray, Kempton, Woolgar, & Hooper (1993) found that speech of depressed mothers expressed more negative affect and was less focused on the infant. In addition, depressive symptoms are associated with reduced sensitivity to infants' distress signals (Donovan et al., 1998). Depressed mothers rated cries as less perceptually salient and as less likely to elicit active caregiving responses, especially at times of greater distress (Schuetze and Zeskind, 2001). Not only does clinical depression affect interaction patterns, mild depression and even experimentally induced maternal depressed mood were found to reduce responsivity (Bettes, 1988; Zekoski, O'Hara, M.W., & Wills, 1986).

In this study we examine the role of the OXTR gene and the influence of depressive symptoms in physiological reactivity to infant crying. In an experimental paradigm, adults' HR responses were measured during the presentation of three sets of cry sounds varying in pitch. We hypothesized that participants with the OXTR GG genotype would display more pronounced HR reactivity across the three cry episodes than participants with the OXTR AA and AG genotype, unless they showed more symptoms of depression that may dampen their reactivity.

Method

Participants

Participants for this study were selected from a larger study investigating the genetic susceptibility to sensitive and harsh caregiving and physiological reactivity in response to infant crying (Out et al., 2010). The original sample consisted of 50 male and 134 female twin pairs who were recruited using the municipal registers of five cities in the western region of the Netherlands, through advertisements and via a website. Zygosity was determined on the basis of questionnaires (Magnus, Berg, & Nance, 1983) and additional genetic analysis of selected polymorphisms. Twin pairs were selected to take part in a future neuroimaging study if they were monozygotic females without children of their own, in good health and without hearing impairments. This resulted in 40 twin pairs for whom the oxytocin receptor gene was determined. The mean age of the selected participants was 27.05 years (SD = 7.55, range 19-47). The majority of the participants were born in the Netherlands and from Caucasian origin (87.5%). Their mean educational level was 3.58 (SD = 0.77) on a scale ranging from 1 (elementary school) to 7 (Bachelor's or Master's degree). Permission for this study was obtained from the local ethics committee and all participants gave informed consent.

Procedure

The twin pairs were invited for a lab session lasting about three hours. They were tested individually in two quiet rooms. The lab session started with several cognitive assessments. Following these assessments and a short break an electrocardiographic device was fitted and their physiological responses were measured during the remaining session. After a one-hour interview, the cry perception task was administered, which lasted about 30 minutes. The participants were told that they would hear infant crying through headphones, and that they had to complete rating scales on-line during the task. At the end of the lab session, participants completed an anxiety questionnaire. They were asked to complete a questionnaire on depressive symptoms at home and returned it by mail within on average two weeks.

Measures

Cry perception task. The cry perception task was administered on a laptop using E-Prime software (Version 1.1; Psychology Software Tools, Inc., PA, USA). Cry sounds were derived from the spontaneous crying of a healthy two-day old, full birth-weight and full term female infant while she was in a supine position in a bassinette, midway between scheduled feedings. The cry was recorded at a sampling rate of 44.1 kHz using a directional microphone held approximately 20 cm from the infant's mouth. A 10-sec portion of the sustained period of crying, containing seven expiratory sounds, was selected for presentation. The durations and peak fundamental frequencies (Peak F_0) of each expiratory component were determined from a digital sound spectrographic display. The frequency of the Peak F_0 was obtained from the power spectrum resulting from a Fast Fourier Transform (FFT) of the 25 msec point at which the fundamental frequency reached its highest point in the expiratory sound. The seven cry expiratory sounds had durations with a mean of 1.055 seconds (range: .545 to 1.899 seconds), and a mean Peak F0 of 452.6 Hz (range: 425.2 to 515.6 Hz). The Peak F_0 of the entire cry was 515$\pm$15 Hz. Two new 10-sec cry stimuli were created by digitally increasing the original cry by approximately 200 and 400 Hz, respectively, resulting in two new 10-sec cry sounds with an overall Peak F_0 of 714.5 Hz (700 Hz Cry) and 895.8 Hz (900 Hz Cry). Changes in the Peak F_0 of these two cries were made with comparable changes in the harmonic structures of the seven cry expirations across the entire 10-sec cry sound segment while holding the temporal components constant.

Participants listened to the cry stimuli that were presented on a constant volume through Sennheiser HD202 headphones. The task started with a baseline condition during which participants were instructed to relax and look at three landscape photographs for six minutes in total. The cry paradigm consisted of two parts. Participants started each part with a practice trial during which the 500 Hz cry was presented. After the practice trial the cry stimuli were presented in three cycles or *episodes* (each consisting of the 500, 700 and 900 Hz stimuli). The order of presentation was random within each cycle. During the first part of the paradigm, the presentation of each stimulus was followed by the collection of a saliva sample which took about one minute. Participants also rated their perception of the cry on four rating scales. During the second part of the task,

participants rated their intended caregiving responses to each cry sound on seven rating scales. No saliva samples were collected in this part. Here we only report on cardiac reactivity during the first part of the cry paradigm. The second part was not included in the present analyses since the procedure was different: the intertrial intervals between the stimuli were shorter and varied across persons.

Depressive symptoms. Participants completed the Dutch version of the Center for Epidemiological Studies Depression Scale (CES-D) (Radloff, 1977). The scale consists of 20 items and measures mood, somatic symptoms, and interpersonal relationships within the last week on a four-point scale based on frequency of occurrence. This scale was found to have high test-retest reliability (r = .51) and high internal consistency (α = .85 for community samples) (Radloff, 1977). The internal consistency in our sample was .88.

Anxiety. The Dutch version of the State-Trait Anxiety Inventory (STAI) (Spielberger, 1983) was used to measure anxiety. The STAI is a 40-item self-report questionnaire that differentiates between the temporary condition of state anxiety and the longstanding quality of trait anxiety. The STAI trait and state both have high test-retest reliability (r = .88, r = .70 respectively) and high internal consistency (α > .89) (Barnes, Harp, & Jung, 2002). In our sample, the internal consistencies for both STAI trait and state were .91.

Cardiac activity. Heart rate was recorded with the Ambulatory Monitoring System (VU-AMS5fs; TD-FPP, Vrije Universiteit, Amsterdam, the Netherlands). The electrocardiogram (ECG) signal was recorded continuously using three disposable pre-gelled Ag-AGCL electrodes (ConMed, New York, USA) that were placed below the right collar bone 4 cm to the right of the sternum, 4 cm under the left nipple and at the lateral right side. The full ECG signal was stored at a 16-bit sampling rate. HR responses were synchronized to the cry sounds using a marker button on the AMS-device. The experimenter pushed the button two seconds before the stimulus was presented, leaving markers that allowed for accurate labeling of each cry sound.

Mean HR was calculated by peak-detection of the R-wave via a Matlab script (Version 7.6.0; MathWorks, MA, USA). The resulting interbeat-intervals were visually inspected by a rater who was unaware of genotype status; in case of irregularities, peak-detection of the raw signal was repeated after using a 5-50 Hz zero phase shifting bandpass filter. Mean HR was calculated for each baseline period and each individual cry episode, resulting in a series of mean HRs for each person. These values were standardized and series from which one or more mean HRs were out of the -3 to +3 range were winsorized (Tabachnik and Fidell, 2001), ensuring that differences within individual series that were characteristic for an individual reactivity pattern remained intact. Baseline levels of HR were averaged across the baseline periods. HR levels during the presentation of the cry stimuli were aggregated across each cry pitch as well as across each of the three cry episodes.

Genotyping. Buccal swabs from the mothers were collected in lysis buffer (100mM NaCl, 10mM EDTA, 10mM Tris pH 8, 0.1 mg/ml proteinase K and 0.5% w/v SDS) until further processing. Genomic DNA was isolated from the samples using the Chemagic buccal swab kit on a Chemagen Module I workstation (Chemagen Biopolymer Technologie AG, Baesweiler, Germany). DNA concentrations were measured using the Quant-iT DNA Assay kit (Invitrogen, Breda, The Netherlands). The average yield was 4 μg of genomic DNA per buccal swab sample. The region of interest from the Oxytocin receptor gene (OXTR rs53576) was amplified by PCR using a forward primer (5'-GCCCACCATGCTCTCCACATC-3') and a reverse primer (5'-GCTGGACTCAGGAGGAATAGGG AC-3'). Typical PCR reactions contained between 10 and 100 ng genomic DNA template, 10 pmol of forward and reverse primers. PCR was carried out in the presence of 5% DMSO with 0.3 U of BioThermAB polymerase (GeneCraft, Munster, Germany) in a total volume of 30 μl using the following cycling conditions: initial denaturation step of 3 min at 95° C, followed by 40 cycles of 30 s at 95° C, 30 s at 60° C, 1 min at 72° C and a final extension step of 3 min at 72° C. To determine the A/G polymorphism, PCR fragments were sequenced using the forward primer and dye terminator chemistry (BigDye v3.1, Applied Biosystems). The genotype distribution (n=10 AA, n=38 AG, n=32 GG) was in Hardy–Weinberg equilibrium, χ^2 (2, n = 80) = 0.07, p = .96. Because of the skewed distribution AA and AG genotypes were combined in the analyses.

Analyses

Data processing. For six participants, physiological measures were lost due to equipment failure or incorrect markers. For four participants, physiological measures were missing during baseline. Missing data imputation was done for these missing values using regression analyses with non-missing physiological measures and age as predictors. Four participants did not complete the CES-D. These missing values were imputed using regression analyses with age and STAI trait as predictors. Depressive symptoms and anxiety are highly correlated (r = .69 in this sample) and they often occur comorbidly (Pollack, 2005).

Statistical analyses. Due to dependency of the data, statistical analyses were done separately for a random half of the sample including one randomly selected sibling from a twin pair. T-tests were performed to examine differences in age, education, depressive symptoms, anxiety, and HR between genotypes GG and AA/AG. To examine the influence of OXTR genotype and depressive symptoms on the development of HR reactivity across the cry paradigm, a repeated measures analysis was performed with HR as the outcome measure, episode (baseline and three episodes) as within-subjects factor, OXTR genotype (GG, AA/AG) as between-subjects factor, and depressive symptoms (mean CES-D) as covariate. State anxiety (mean STAI state) was included as a covariate in order to control for temporary mood during the lab session. Degrees of freedom were corrected using Greenhouse-Geisser and Huynh-Feldt estimates of sphericity if Mauchly's test indicated that the assumption of sphericity was violated; main effects were based on pooled error. All analyses were repeated for the second half of the sample.

Results

Table 1 presents the descriptive statistics of education, depressive symptoms, trait and state anxiety and HR during baseline and the three cry episodes for each genotype. The genotype groups did not differ on any of these variables.

The repeated measures analysis did not show a significant effect of state anxiety, $F(1,31) = 0.02$, $p = .88$; we therefore excluded the variable from further analyses. There was a significant main effect of episode ($F(2.38, 76.00) = 9.73$, $p < .01$, partial $\eta^2 = .23$, $\varepsilon = .79$). Contrasts revealed that HR was significantly higher during the first cry episode ($F(1,32) = 4.27$, $p = .05$, partial $\eta^2 = .12$), the second cry episode ($F(1,32) = 19.65$, $p < .01$, partial $\eta^2 = .38$) and the third cry episode ($F(1,32) = 17.67$, $p < .01$, partial $\eta^2 = .36$) compared to baseline. There was no significant effect of OXTR genotype ($F(1,32) = 0.77$, $p = .39$) or depressive symptoms ($F(1,32) = 0.07$, $p = .79$), nor any interaction between OXTR genotype and depressive symptoms ($F(1,32) = 0.72$, $p = .40$) and between depressive symptoms and cry episode ($F(2.38, 76.00) = 2.07$, $p = .12$, $\varepsilon = .79$).

However, there was a significant three-way interaction between cry episode, OXTR genotype and depressive symptoms ($F(2.38, 76.00) = 4.11$, $p = .02$, partial $\eta^2 = .11$, $\varepsilon = .79$). Participants with the GG genotype *and* low scores on CES-D had the largest increase in HR across the cry paradigm, especially during the second cry episode ($F(1,32) = 11.15$, $p < .01$, partial $\eta^2 = .26$) and the third cry episode ($F(1,32) = 7.37$, $p = .01$, partial $\eta^2 = .19$). The three-way interaction is illustrated in Figure 1, with depressive symptoms dichotomized into low (Figure 1a, $M = 3.66$, $SD = 2.64$) versus high (Figure 1b, $M = 13.02$, $SD = 6.04$) CES-D scores using a median split (median = 8.12). There was a significant main effect of episode for participants with the AA/AG genotype and low CES-D scores ($F(3, 36) = 6.73$, $p < .01$, partial $\eta^2 = .36$) or high CES-D scores ($F(3,24) = 10.96$, $p < .01$, partial $\eta^2 = .58$), as well as for the participants with the GG genotype and low CES-D scores ($F(3,15) = 4.69$, $p = .02$, partial $\eta^2 = .48$), but not for participants with the GG genotype and high CES-D scores ($F(3,21) = 2.75$, $p = .07$). To ensure that the effects were not confounded by population stratification, analyses were repeated after exclusion of participants who were not from Caucasian origin ($n = 5$). The three-way interaction between cry episode, OXTR genotype and depressive symptoms remained significant ($F(2.40, 69.58) = 5.70$, $p < .01$, partial $\eta^2 = .16$, $\varepsilon = .80$).

Analyses were repeated for the second half of the twin pair sample. Again state anxiety was excluded from the analyses, because it did not show a significant effect on HR ($F(1,33) = 0.06$, $p = .81$). The main effect of episode was significant ($F(2.05, 69.64) = 15.39$, $p < .01$, partial $\eta^2 = .31$, $\varepsilon = .68$). Contrasts revealed that HR was significantly higher during the first cry episode ($F(1,34) = 13.75$, $p < .01$, partial $\eta^2 = .29$), the second cry episode ($F(1,34) = 25.40$, $p < .01$, partial $\eta^2 = .43$) and the third cry episode ($F(1,34) = 22.63$, $p < .01$, partial $\eta^2 = .40$) compared to baseline. Similar to the first half of the twin pair sample, there was no significant effect of OXTR genotype ($F(1,34) = 1.67$, $p = .21$) or depressive symptoms ($F(1,34) = 1.12$, $p = .30$), nor any interaction between OXTR genotype and depressive symptoms ($F(1,34) = 3.96$, $p = .06$) and between depressive symptoms and cry episode ($F(2.05,69.64) = 2.90$, $p = .06$, $\varepsilon = .68$). As in the first sample, the three-way

Table 1 Means and standard deviations for CES-D, STAI state, STAI trait, education, and HR during baseline and three cry episodes for the OXTR GG and AA/AG genotypes.

	Sample 1								Sample 2							
	Genotype GG			Genotype AA/AG					Genotype GG			Genotype AA/AG				
Variable	M	SD	Range	M	SD	Range	*t*	*p*	M	SD	Range	M	SD	Range	*t*	*p*
Education[b]	3.85	0.67	2-5	3.57	0.93	3-5	-0.92	0.36	3.64	0.33	2-5	3,43	0.79	2-5	-0.84	0.41
CES-D[a]	8.67	3.05	4-14	7.70	8.06	0-34	-0.43	0.67	10.37	7.51	0-30	8.86	6.25	1-25	-0.67	0.51
STAI state[a]	1.69	0.28	1.25-2.15	1.71	0.51	1.05-3.55	0.12	0.91	1.54	0.30	1.00-2.05	1.58	0.39	1.00-2.35	0.33	0.74
STAI trait[a]	1.79	0.39	1.20-2.80	1.82	0.55	1.15-3.55	0.18	0.38	1.79	0.38	1.20-2.45	1.68	0.34	1.25-2.50	-0.88	0.38
HR Baseline[a]	67.70	6.74	58.18-80.55	67.08	9.85	47.50-92.54	-0.21	0.84	65.25	8.90	49.67-84.46	68.67	9.41	51.58-85.47	1.12	0.27
HR Episode 1[a]	70.39	8.65	57.87-82.55	69.53	9.64	49.49-96.06	-0.27	0.79	67.91	10.51	50.77-86.46	70.36	8.76	51.62-83.30	0.78	0.44
HR Episode 2[a]	70.85	7.64	59.32-80.94	70.78	10.61	51.63-97.84	-0.02	0.98	70.09	10.72	53.97-91.03	71.22	8.28	53.25-85.02	0.37	0.72
HR Episode 3[a]	73.23	10.00	58.21-89.07	70.97	10.01	51.26-97.88	-0.69	0.50	71.36	11.87	53.18-92.02	72.49	7.92	53.27-86.13	0.35	0.73

Note. Sample 1: [a]*n*=36 (*n*=14 GG, *n*=22 AA/AG), [b]*n*=34 (*n*=13 GG, *n*=21 AA/AG), Sample 2: [a]*n*=38 (*n*=15 GG, *n*=23 AA/AG), [b]*n*=36 (*n*=15 GG, *n*=21 AA/AG)

Figure 1a.

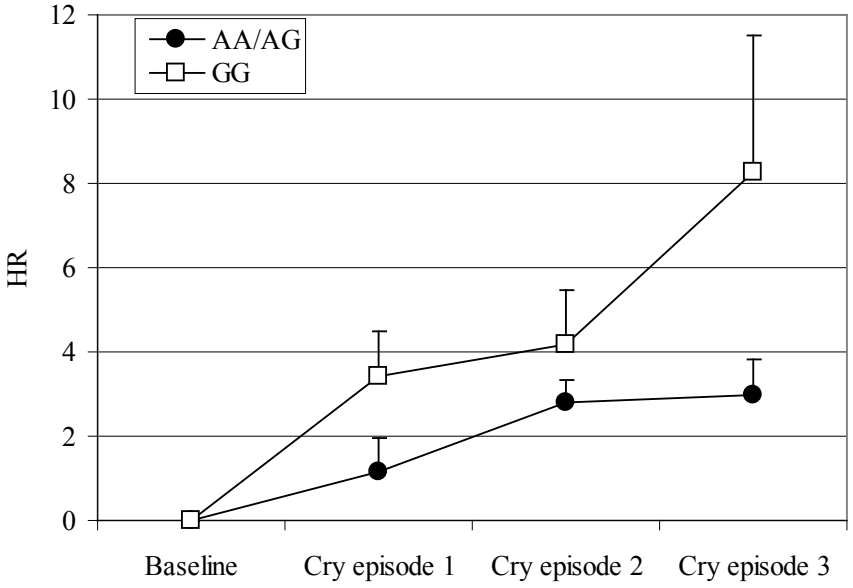

Figure 1b.

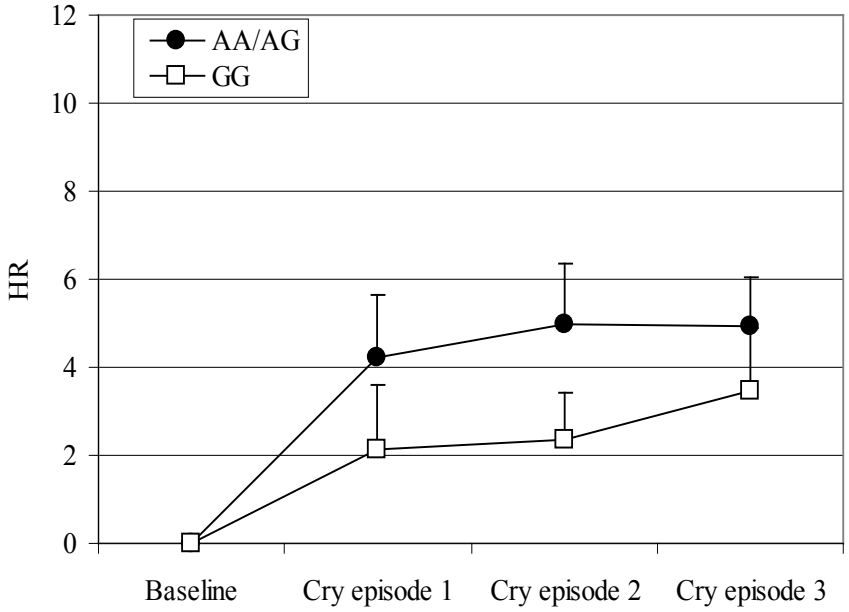

Figure 1 Heart Rate reactivity across the three cry episodes for participants in the first half of the twin pair sample with OXTR AA/AG or GG and low (Figure 1a, *n*=13 AA/AG, *n*=6 GG) and high (Figure 1b, *n*=9 AA/AG, *n*=8 GG) levels of depression.

Figure 2a.

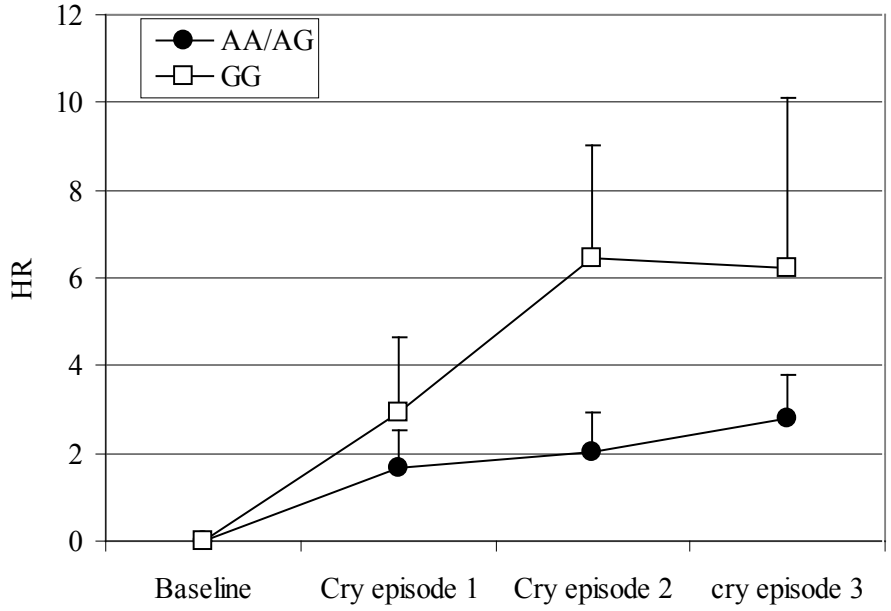

Figure 2b.

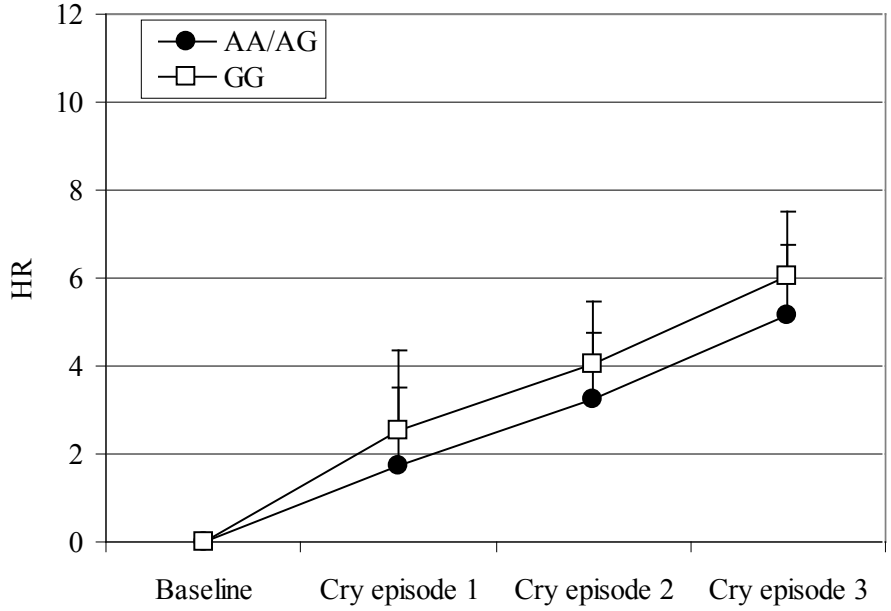

Figure 2 Heart Rate reactivity across the three cry episodes for participants in the second half of the twin pair sample with OXTR AA/AG or GG and low (Figure 2a, *n*=13 AA/AG, *n*=5 GG) and high (Figure 2b, *n*=10 AA/AG, *n*=10 GG) levels of depression.

interaction of episode, OXTR and depressive symptoms was significant (F(2.05, 69.64)= 3.77, p = .03, partial η^2 = .10, ε = .68). Again, participants with the GG genotype and low scores on CES-D had the largest increase in HR across the cry paradigm, especially during the second cry episode (F(1,34) = 6.06, p = .02, partial η^2 = .15) and the third cry episode (F(1,34) = 5.49, p = .03, partial η^2 = .14), thus replicating the results in the first half of the sample. The three-way interaction in the second half of the twin pair sample is illustrated in Figure 2. As shown in Figure 2, participants with the GG genotype with low CES-D scores show an earlier increase in heart rate reactivity (during cry episode 2) compared to other participants. Mean CES-D score in the group with low depressive symptoms was 4.24 (SD = 2.33) and 14.15 (SD = 5.83) in the group with high depressive symptoms. There was a significant main effect of episode for participants with the AA/AG genotype and low CES-D scores (F(3,36) = 4.10, p = .01, partial η^2 = .26) or high CES-D scores (F(3,27) = 5.35, p < .01, partial η^2 = .37), as well as for the participants with the GG genotype and low CES-D scores (F(3,12) = 3.43, p = .05, partial η^2 = .46) or high CES-D scores (F(3,27) = 8.73, p < .01, partial η^2 = .49). After exclusion of the non-Caucasian participants (n = 5) the three-way interaction between cry episode, OXTR genotype and depressive symptoms remained significant (F(2.08, 60.42) = 5.60, p < .01, partial η^2 = .16, ε = .69).

Discussion

Participants with the OXTR GG genotype displayed more pronounced physiological reactivity across the cry paradigm than participants with the AA or AG genotype. Similar to Out et al. (2010) the results point to genetic effects on increased HR to repeated infant cries, and we documented the role of the OXTR genotype in this sensitization response. However, participants with the GG genotype did *not* show more pronounced physiological reactivity when they had more symptoms of depression. Thus, adults with the GG genotype became more sensitized to repeated infant cries than participants with the potentially less effective variants of the oxytonergic system gene, unless they showed more symptoms of depression. Our findings cannot be explained by an association between depressive symptoms and OXTR genotype, since depressive symptoms and OXTR genotype were unrelated. We replicated the results in the other half of the twin sample.

Recently, Bakermans-Kranenburg and Van IJzendoorn (2008) found that mothers with OXTR GG genotype were more sensitive to their toddlers' signals than mothers with the AA or AG genotype. More pronounced physiological reactivity might form the basis for maternal sensitivity, because high arousal has been shown to lead to quick responding to the infant (Del Vecchio et al., 2009), though it should be mentioned that very strong physiological reactivity to infant cries may also be associated with harsh responses (Crowe and Zeskind, 1992). Several studies have demonstrated the important role of oxytocin in maternal behavior (e.g. Feldman et al., 2007). Oxytocin prompts affiliative behavior as a response to stress, such as tending and protective responses to infants (Taylor, 2006). At the same time, depressive symptoms have been shown to dampen

maternal sensitivity (Donovan et al., 1998; Murray et al., 1993; Murray et al., 1996). To our knowledge, this is the first study to suggest effects of OXTR genotype in combination with depressive symptoms on physiological reactivity to infant crying.

The current study is limited in several ways. First, our sample size is relatively small compared to other studies that examine the association between sensitivity and depressive symptoms or genotype. This resulted in small genotype group sizes and the necessary combination of the OXTR AA and AG genotypes in the analyses (similar to Bakermans-Kranenburg and Van IJzendoorn, 2008). However, our results were replicated in the other half of the twins, consolidating our findings in the first half. Of course, the second sample was genetically and otherwise associated with the first sample which makes it easier to replicate although assessments were conducted independently. Second, the participants did not have a diagnosis of clinical depression. Our findings indicate that more depressive symptoms dampen sensitivity to infant crying even in a subclinical population. Sensitivity to infant crying might be reduced even more in adults who are diagnosed with clinical depression, as shown by Schuetze and Zeskind (2001). Furthermore, it should be mentioned that we focused on variants of the oxytocin receptor gene that have not yet been shown to be functional. The Bakermans-Kranenburg and Van IJzendoorn (2008) study was one of the first suggesting functional implications for GG vs AG and AA variants of OXTR. In previous studies, variations in OXTR have been related to autism (Wu et al., 2005), indicating genetic vulnerability to autism in carriers of the A allele. The processes linking variants of the OXTR gene to actual oxytocin levels in humans have however not yet been clarified. Carter et al. (2007) detected variations of oxytocin levels in saliva samples as a function of lactation and massage. Measurement of oxytocin in saliva may be a promising future method to test the association between oxytocin and sensitivity to infant crying more directly.

Further research is needed to investigate how OXTR genotype and depressive symptoms affect behavior of typical, non-twin parents instead of twin adults who did not (yet) have children of their own. In the current study we focused on physiological measures of sensitivity to infant cries. Elsewhere, we documented that parents and non-parents did not differ in the heritability of heart rate while listening to infant cries (Out et al., 2010). Since physiological reactivity to infant cries is associated with responsivity to the infant (Del Vecchio et al., 2009), OXTR genotype and depressive symptoms are expected to influence parent-child interaction as well. In addition, future studies may target the processes underlying the association between OXTR genotype, depressive symptoms and physiological reactivity to infant crying. OXTR genotype might affect neural networks involved in emotional processes, such as the perception of infant cries. In a future neuroimaging study we aim at clarifying how OXTR genotype and depression affect these neurological processes.

In conclusion, the current study is the first to highlight the role of OXTR genotype in combination with depressive symptoms on physiological reactivity to repeated infant crying. The findings support previous results of decreased sensitivity in depressed mothers and increased sensitivity in the OXTR GG

genotype. Adults with the OXTR GG genotype showed more pronounced physiological reactivity to repeated infant cries than adults with the potentially less efficient variant of the oxytonergic system gene, but depressive symptoms may suppress the effect of the OXTR GG genotype.

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