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Emotion perception in motherhood and infancy: The role of breastfeeding experience and the oxytocin system

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Kathleen Marie Krol,
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Dean: Prof. Dr. Klaus Fiedler
Advisor: Prof. Dr. Tobias Grossmann

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Table of contents

List of scientific publications[1]

I.
Krol, Kathleen M., Kamboj, Sunjeev K., Curran, H. Valerie, & Grossmann, Tobias (2014). Breastfeeding experience differentially impacts recognition of happiness and anger in mothers. *Scientific Reports*, *4*, 7006.

II.
Krol, Kathleen M., Rajhans, Purva, Missana, Manuela, & Grossmann, Tobias (2015). Duration of exclusive breastfeeding is associated with differences in infants' brain responses to emotional body expressions. *Frontiers in Behavioral Neuroscience*, *8*, 459.

III.
Krol, Kathleen M., Monakhov, Mikhail, Lai, Poh San, Ebstein, Richard P., Grossmann, Tobias (2015). Genetic variation in CD38 and breastfeeding experience interact to impact infants' attention to social eye cues. *Proceedings of the National Academy of Sciences USA, 112*(39), E5434-E5442.

[1] For the consistency of the current thesis, all original publications have been adapted into APA formatting style and American English where necessary.

List of figures

List of tables

Abbreviations

ASD = autism spectrum disorder

BNST = bed nucleus of the stria terminalis

CD38/*CD38* = Cluster of Differentiation 38/CD38 gene

CRH = corticotropin-releasing hormone

DHA = docosahexaenoic acid

DNA = deoxyribonucleic acid

EBF = exclusive breastfeeding

EEG = electroencephalography

ERP = event-related potential

HPA = hypothalamic-pituitary-adrenal axis

LCPUFA = long-chain polyunsaturated fatty acid

LS = lateral septum

MPOA = medial preoptic area

mRNA = messenger ribonucleic acid

Nc = negative central component

OXTR = oxytocin receptor gene

PVN = paraventricular nucleus

SNP = single-nucleotide polymorphism

SON = supraoptic nucleus

Part I. Introduction

Chapter 1.
General Introduction

1.1 Preamble

The ability to distinguish between emotional expressions is a crucial building block for proper, normative social functioning. By making meaning from others' nonverbal expressions, one can infer affective states and intentions, predict future behavior, and plan one's own behavior accordingly. Differences arise both between and within individuals in their emotion processing abilities, and oftentimes, atypical emotion perception is associated with maladaptive functioning. It is unclear what factors may mediate the perception of emotion, giving rise to the differences observed in children and adults. There is strong evidence to suggest that infants develop this skill at a very early age, within the first year of life. It is therefore of great importance to investigate influential factors on emotion perception in infancy. One compelling candidate is the oxytocin system. We know that oxytocin can mediate emotional processing in adults. Very fittingly, infants vary immensely with regard to early oxytocin exposure. Lactation is a highly conserved element of maternal care, characterized by tremendous levels of oxytocin in both mothers and offspring. In the current thesis, I have thus assembled these reflections into a single question: Does the experience of breastfeeding, through its interaction with the oxytocin system, impact emotion perception?

1.2 Built to be social: The development of emotion perception

Humans are ultra-social animals (Tomasello, 2014). As adults, it may seem trivial that we are able to interact with and understand one another, but many developmental building blocks were required to get us to the communicative and expressive people we are today. From birth,

infants' perceptual systems are tuned to social stimuli. Newborns are already sensitive to the structural elements of faces; that is, two eyes on the top, and a nose and mouth below (Morton & Johnson, 1991). They show significantly longer looking times to "face-like" images when compared to scrambled images (Goren, Sarty, & Wu, 1975; Johnson, Dzirawiec, Ellis, & Morton, 1991). Even when the superficial aspects of a face (i.e., eyes) are replaced by squares, newborns nevertheless look longer at these squares when presented in a face-like array (Valenza, Simion, Cassia, & Umiltà, 1996) (please see Figure 1.1 for stimulus examples from this seminal research). This rapid face detection is likely controlled by subcortical, automatic, and highly conserved processes mediated by the superior colliculus (Johnson, Senju, & Tomalski, 2015). Upon detection of faces, this subcortical route activates relevant cortical regions for attention such as the orbitofrontal cortex, lateral occipital cortex, and fusiform gyrus. With increased experience, infants rapidly acquire and retain information on the visual characteristics of faces seen most frequently in their natural environment, thus developing species-specific and person-specific preferences (Johnson & Morton, 1991; Johnson et al., 2015). The cortical activation to face stimuli increases by the hour in newborn infants, such that this "social tuning" continues to strengthen (Farroni et al., 2013).

The visual acuity of the infant reaches adult-like levels around six months of age; at this time they are able to perceive fine details of a face. This increased acuity most certainly aids infants as they begin to discriminate between meaningful emotional expressions (Gwiazda, Bauer, & Held, 1989; Hainline & Abramov, 1992). Emotional expressions have a long phylogenetic history and a strong biological basis, as they provide an exceptionally effective way for one to communicate one's own internal state (Ekman, 1973; Goos & Silverman, 2002). The efficient assessment of another's affective state enables the prediction of future behavior of others, as well as the planning of one's own behavior on the basis of these predictions. Distinguishing between these emotional cues at an early age is crucial as the infant begins to experience the world. Learning these signals serves the adaptive purpose of allowing them to safely explore the

environment while appropriately avoiding harmful situations. For example, an angry face communicates the presence of an imminent threat, while a smile is a signal of approach and functions as a facilitator of social contact (Seidel, Habel, Kirschner, Gur, & Derntl, 2010; Stins et al., 2011).

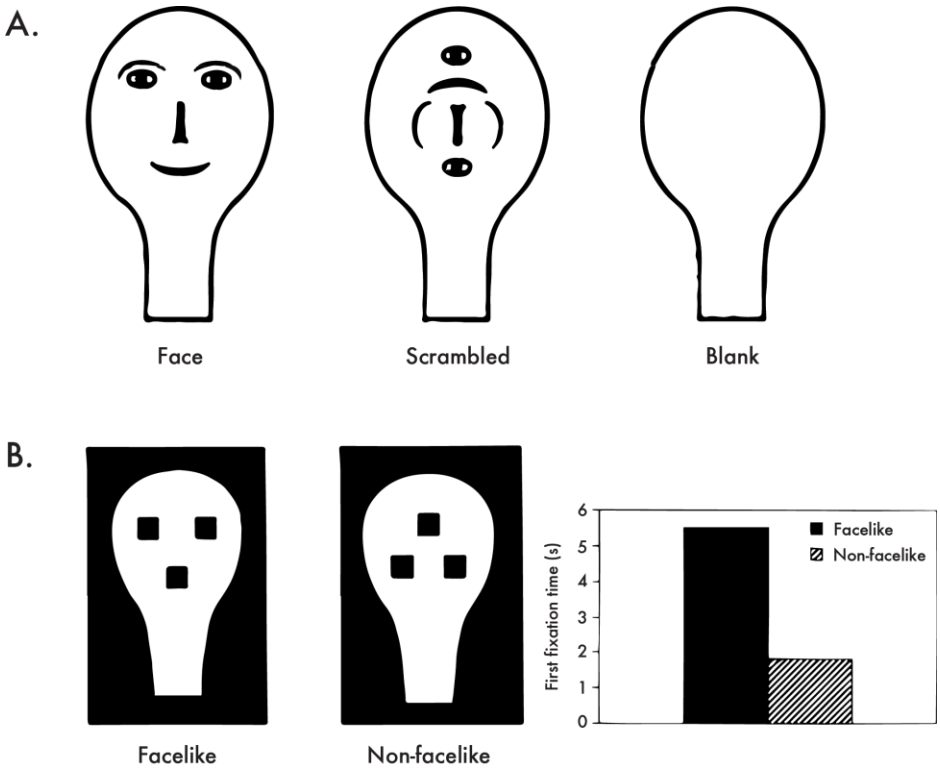

Figure 1.1 Newborn infants prefer facelike stimuli. A) Stimuli used in original study by Johnson and colleagues (1991). Within the first hours of birth, infants orient more towards the "face" stimulus than either "scrambled" or "blank". B) Stimuli used by Valenza and colleagues (1996), in which infants looked longer at the "facelike" stimulus than the "non-facelike", suggesting that even when the superficial features of a face are removed, infants are still tuned to the general upright orientation of a human face. All figures adapted with permission.

Several emotional facial expressions have been shown to be universally recognized across cultures. While original work indicated that there are six basic facial emotions (anger, happiness, fear, surprise, disgust, and sadness) (Ekman, 1992), more recent work suggests that there are only four emotion categories readily-recognized across cultures: happiness, sadness, fear/surprise (fast-approaching danger), and disgust/anger (stationary danger) (Jack, Garrod, & Schyns, 2014). Healthy adults are promptly able to recognize and categorize emotional expressions from face,

voice, and body (Atkinson, Dittrich, Gemmell, & Young, 2004; Sauter, Eisner, Ekman, & Scott, 2010). There is behavioral, neural, and physiological evidence to suggest that infants become able to distinguish and categorize emotional expressions within the first year of life (Grossmann, 2013). This has been found across several domains, including positive and negative facial expressions (Kotsoni, de Haan, & Johnson, 2001; Peltola, Leppänen, Maki, & Hietanen, 2009), vocal expressions (Grossmann, Striano, & Friederici, 2005), body expressions (Missana, Atkinson, & Grossmann, 2015; Missana, Rajhans, Atkinson, & Grossmann, 2014) and even for tactile (pleasant touch) information (Fairhurst, Löken, & Grossmann, 2014).

Not unlike most human capabilities, there is considerable variation between individuals in emotional processing. It is of great importance not only to understand the developmental underpinnings of emotion perception but also to identify factors that may contribute to enhanced or reduced responding to emotionally-valenced information. In the following thesis, I will examine possible experiential and genetic mechanisms which may contribute to individual differences in emotion perception. Specifically, I will explore breastfeeding experience and genetic variation within the oxytocin system in mothers and infants in order to gain a better understanding of how certain experiences can impact emotion perception.

1.3 The case for breastfeeding: Why might it impact social development?

1.3.1 Maternal care and child development

The level of maternal care one receives in early life can have profound effects on brain development and behavior. Studies with animals have investigated the role of maternal care in programming the behavior and physiological stress responses in their offspring. For example, high maternal care from rat dams (greater arched back nursing, licking and grooming) impacts fearful behavior, exploration, and play in rat pups (Fish et al., 2004; Kappeler & Meaney, 2010; Meaney, 2010; Szyf, Weaver, & Meaney, 2007). Pups with high maternal care mothers exhibit

attenuated hypothalamic-pituitary-adrenal (HPA) axis reactivity to stress, and greater concentrations of glucocorticoid receptor expression in the hippocampus allowing for a quicker shut-down of stress responses through negative feedback (Liu et al., 1997). Moreover, female pups that received high maternal care exhibit high maternal care to their own young (Champagne, Francis, Mar, & Meaney, 2003). This generational transfer of behavior is also observed in cross-fostering studies in which rat pups are not raised by their biological mothers, suggesting that individual differences in maternal care are not necessarily genetic, but instead are likely to be epigenetic (Champagne, 2008; Champagne & Curley, 2009; Francis, Diorio, Liu, & Meaney, 1999). Epigenetics refers to processes that facilitate or turn down the expression of particular genes as a result of developmental or experiential events. Thus, early experience can alter gene expression and, in turn, behavior.

The notion that maternal care (or lack thereof) can program the physiology and behavior of offspring has also been documented in humans. For example, orphaned infants raised without their mothers in institutionalized care tend to show impaired health, cognitive, and neural outcomes (Gee et al., 2013; Roeber, Gunnar, & Pollak, 2014; Tottenham et al., 2010; Wiik et al., 2011). In these contexts, caregivers rotate in shifts and are in charge of a high number of children, sometimes as high as 20 children to one caregiver (Gunnar, Bruce, & Grotevant, 2000; Gunnar, Van Dulmen, & Team, 2007). While research on institutionalized orphan children is important, there is also considerable variation of maternal care in normative populations that might be linked to individual differences in social and emotional functioning. For example, adults who rate their mothers as having provided low maternal care have been shown to exhibit significantly higher cortisol upon awakening than those who reported receiving high maternal care (Engert, Efanov, Dedovic, Dagher, & Pruessner, 2011). Moreover, perceived maternal care has also been shown to impact cortisol levels in response to social stress in adulthood (Engert et al., 2010). Thus, maternal care experience may particularly target the stress response, with less care being associated with higher stress reactivity.

One major question that arises from such retrospective self-report studies in adults is what elements of maternal care play a critical role in the development of differences in social and emotional functioning of the individual. Existing theories on parenting and attachment have attempted to deal with this question by categorizing the *quality* of maternal care and its effects on the attachment relationship between mother and infant (Ainsworth & Bowlby, 1991; Baumrind, 1966, 1967; Maccoby & Martin, 1983). However, another vital element of human maternal care deeply rooted in our biology, characteristic of all mammalian species, and greatly neglected in the investigation of social and emotional processes in mothers and infants, is breastfeeding. Breastfeeding is a complex and dynamic physiological and psychosocial process that critically depends on oxytocin (Dawood, Khandawood, Wahi, & Fuchs, 1981). While deeply rooted in our biology, breastfeeding behavior is highly variable among mothers in terms of duration (Kanazawa, 2015). Thus, studying the duration of breastfeeding and the likely effects on the oxytocin system allows us to assess naturally occurring variation in maternal behavior and its relation to social and emotional responding in mothers and infants.

1.3.2 Breastfeeding as a vital element of maternal care

Lactation is a process characteristic of all mammalian species. The milk of each species is tailored specifically to the needs of the young (Hinde, 2013). It is the result of millennia of selective pressure to be an optimal nutrient delivery system, involved in supplying all essential nutrients in the correct amounts to the infant (Hinde & German, 2012) (Figure 1.2). Species differ extensively in their lactation strategies (Lefevre, Sharp, & Nicholas, 2010). For example, due to the icy conditions of the North Atlantic, hooded seal pups are only nursed for four days and the milk contains the highest fat concentration known among mammals (Iverson, Oftedal, Bowen, Boness, & Sampugna, 1995). In contrast, primates have a much longer period of lactational investment, and thus, our milk contains less fat (Hinde & German, 2012).

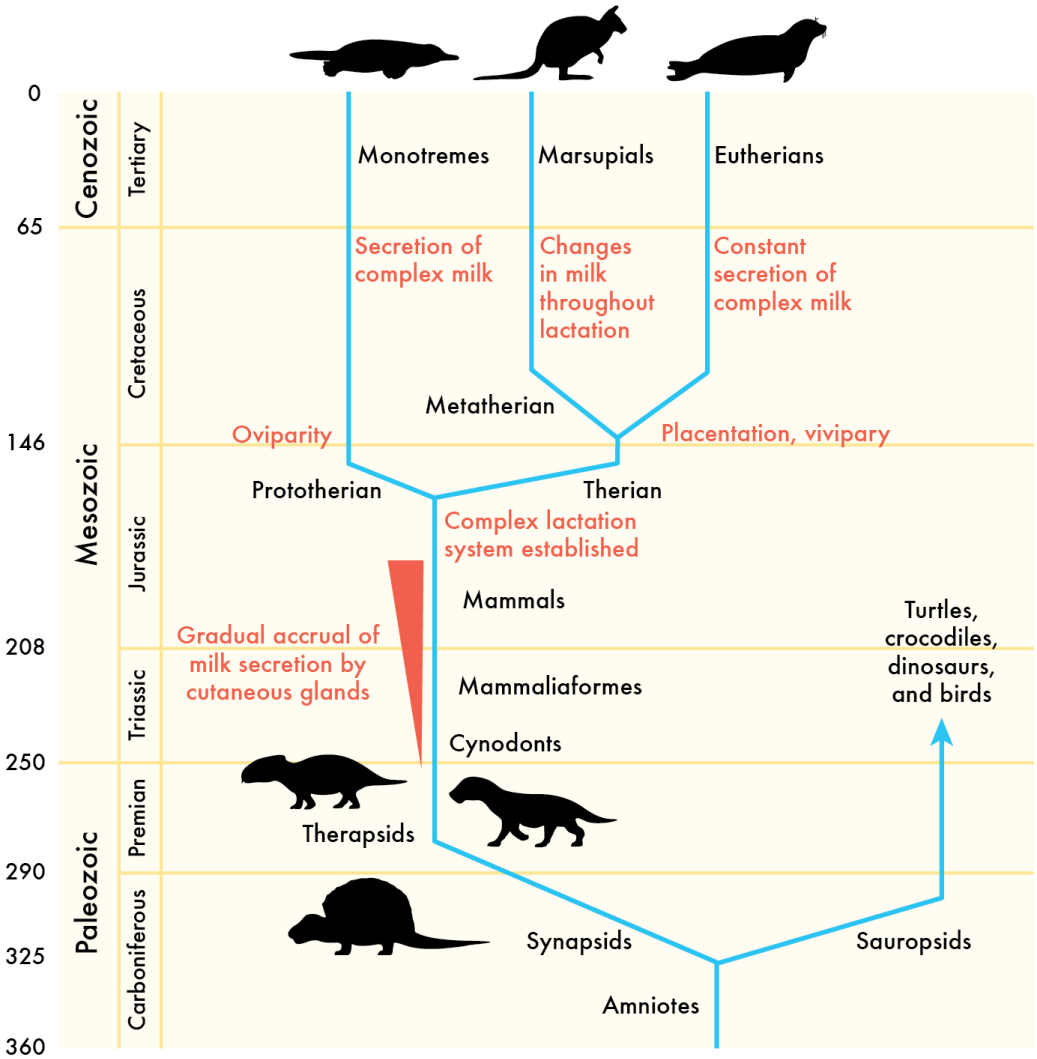

Figure 1.2 Lactation is a characteristic trait of all mammals and is highly conserved. Figure adapted from Lefevre et al. (2010), with permission.

While being the critical source of nutrition to the infant, there is a general consensus that breastfeeding has other beneficial effects. Milk is an immensely complex cocktail of oligosaccharides, hormones, vitamins and minerals, bacteria, immunoglobulins, and antioxidants. Indeed, scientists are still discovering components of human milk (Xavier, Rai, & Hegde, 2011). Specific micro-constituents of milk likely contribute to cognitive, neurobiological, and immune development in infants (Hinde, 2009). A wealth of literature has highlighted the immense impact of breastfeeding on the immune system of the developing infant (Fallani et al., 2010; Hanson & Korotkova, 2002; Hasselbalch, Jeppesen, Engelmann, Michaelsen, & Nielsen, 1996; Kramer et

al., 2003). For the purposes of this thesis, I will provide an overview of more recent discoveries of cognitive and neural outcomes in infants, as well as outcomes in mothers most relevant to the scope of my research question.

1.3.2.1 Breastfeeding and cognitive and neural development in offspring

A substantial body of literature from around the globe has noted a link between breastfeeding and cognitive development later in life (Daniels & Adair, 2005; Kramer et al., 2008; Ludington, Hadeed, & Anderson, 1991; Mortensen, Michaelsen, Sanders, & Reinisch, 2002; Oddy, 2002; Oddy et al., 2004). Longitudinal, prospective designs are particularly useful in gauging the potential link between breastfeeding and cognitive development. For example, greater levels of breastfed meals and exclusive breastfeeding duration during the first year of life positively relate to measures of the Bayley Scales of Infant Development, including memory, early language skills, and the assessment of fine and gross motor development at 14 months of age (Guxens et al., 2011) and, in a different cohort, 18 months of age (Leventakou et al., 2015). These developmental advantages have been shown to persist into toddlerhood. For example, Bernard et al. (2013) assessed cognitive and motor development in two- and three-year old children and found that breastfeeding experience was positively associated with cognitive development as measured by the Communicative Development Inventory and Ages and Stages Questionnaire. In particular, problem-solving abilities in toddlers were strongly associated with duration of exclusive breastfeeding. Another longitudinal study using the Wechsler Intelligence Scale for Children to measure cognitive skills reported cognitive gains at the age of one year and even further at the age of seven years as a function of prolonged exclusive breastfeeding duration during infancy (Jedrychowski et al., 2012). Recent findings from a cohort of two consecutive generations suggest that each month of breastfeeding in infancy increased IQ by .16 points in childhood, even after controlling for the IQ of the mothers (Kanazawa, 2015).

There is also evidence to demonstrate that breastfeeding experience during infancy impacts cognitive abilities well into adulthood. For example, Mortensen and colleagues (2002)

investigated cognitive skills in two different cohorts, using two different intelligence tests. At 18 years of age, longer durations or breastfeeding were positively associated with performance on the Borge Priens Prove, a standard Danish intelligence test. In a separate cohort, duration of breastfeeding was significantly associated with performance on the Wechsler Adult Intelligence Scale at 27 years of age (Mortensen et al., 2002). Similarly, recent findings from a Brazilian cohort revealed that the duration of exclusive breastfeeding was positively associated with an increased IQ, educational attainment, and income at 30 years of age (Victora et al., 2015). Strikingly, this association holds until much later in the lifespan. Specifically, there is work to show that breastfeeding duration is positively associated with reading ability at 53 years, as measured by the National Adult Reading Test (Richards, Hardy, & Wadsworth, 2002). It is important to highlight that the aforementioned studies controlled for a large range of potentially confounding variables, including but not limited to parental education, employment, income, maternal age, method of delivery, cigarette consumption during pregnancy, and birth weight.

While it is generally accepted that breastfeeding exposure facilitates cognitive development, the exact mechanism is unclear. One possible mechanism relates to the long-chain polyunsaturated fatty acids (LCPFAs), which are present in human milk and very frequently absent in formula (Drover, Hoffman, Castaneda, Morale, & Birch, 2009). One major LCPFA is docohexaenoic acid (DHA), which is necessary for neurodevelopment by contributing to healthy neuronal growth, repair, and myelination (Guesnet & Alessandri, 2011). Myelination predominately occurs postnatally, within the first 18 months of life (Cockburn, 2003; Deoni et al., 2013). Infants produce a small quantity of DHA during the first two weeks of life, but are then unable to produce sufficient amounts on their own until about six months of age (Cockburn, 2003). This opens the possibility of a window in development in which the brain may be particularly sensitive to experience. Indeed, many neural outcomes, including total brain volume, cortical thickness, and particularly white matter volume, have been found to be increased by a prolonged duration of breastfeeding experience (Deoni et al., 2013; Isaacs et al., 2010;

Kafouri et al., 2013). For example, Deoni and colleagues (2013) investigated white matter maturation from 10 months to four years of age and found a positive association between the development of white matter and the duration of exclusive breastfeeding. This study shows that breastfeeding-related increases in white matter occurred in regions that typically mature later, including frontal and temporal regions, as well as pathways commonly associated with higher-order cognition and socio-emotional functioning, including the superior longitudinal fasciculus, occipitofrontal fasciculus, peripheral aspects of the internal capsule and corticospinal tracts (Deoni et al., 2013). Taken together, these findings postulate that elements of breast milk itself may contribute to neural maturation at a young age, particularly in regions that are important for social and cognitive development (Grossmann, 2015).

1.3.2.2 Effects of breastfeeding in mothers

Breastfeeding has been reported to impact mood and stress reactivity in breastfeeding mothers (Heinrichs, Neumann, & Ehlert, 2002). However, whether these effects are as enduring as the cognitive effects across development discussed above remains unclear. Regardless, exclusive breastfeeding is thought to play a vital role in facilitating the bond between mother and child by promoting positive affect and maternal sensitivity (Brandt, Andrews, & Kvale, 1998; Kennell & McGrath, 2005; Zetterström, 1999). For example, exclusively breastfeeding mothers exhibit greater brain activation in several limbic regions when listening to their own infant's cries as compared to exclusive formula feeders (Kim et al., 2011). More generally, exclusive breastfeeding has been shown to have an impact on the overall well-being of mothers. Specifically, breastfeeding mothers report lower negative moods, anxiety, and stress than formula-feeders (Groër, 2005). This subjective report has been qualified by objective measures that suggest an anxiolytic effect of breastfeeding. For example, breastfeeding mothers have stronger cardiac vagal tone modulation, reduced blood pressure, and reduced heart rate reactivity than formula feeders, indexing a calm and non-anxious physiological state (Hahn-Holbrook, Holt-Lunstad, Holbrook, Coyne, & Lawson, 2011; Mezzacappa, Kelsey, & Katkin, 2005).

Moreover, there is evidence to show that breastfeeding mothers have a reduced cortisol response when faced with social stress (Heinrichs et al., 2002). Breastfeeding produces a cascade of endocrinological and physiological changes, which, in turn, may mediate the aforementioned outcomes in terms of positive affect and stress and anxiety reduction in mothers (Heinrichs et al., 2002). The physiological processes underlying lactation will be discussed extensively in the next section.

1.3.3 Lactation and milk let-down

During the peripartum period, the maternal brain goes through a wide variety of molecular and morphological changes in preparation for lactation (for a review, see Hillerer, Jacobs, Fischer, & Aigner, 2014). Notably, there is increased gene expression and release of oxytocin in the hypothalamic paraventricular (PVN) and supraoptic (SON) nuclei and in limbic regions of the brain (Kendrick, Keverne, Hinton, & Goode, 1992; Landgraf, Neumann, Russell, & Pittman, 1992; Zingg & Lefebvre, 1988). Consistent with this, there is an increase in the expression of oxytocin receptor gene (*OXTR*) in the PVN, SON, as well as regions vital for maternal care such as the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST), and the lateral septum (LS) (Insel, 1986; Meddle, Bishop, Gkoumassi, van Leeuwen, & Douglas, 2007). In contrast, corticotropin-releasing hormone (CRH) expression, production, and release are markedly reduced, signifying a reduction in the HPA axis and thus, stress reactivity (Toufexis, Tesolin, Huang, & Walker, 1999). Prolactin levels begin increasing toward the end of pregnancy, such that levels are already 10 to 20 times higher than normal amounts. Prolactin initiates milk production; continued nursing (and oxytocin release) stimulates the release of prolactin, thus the continued synthesis of milk (Ostrom, 1990).

Similar to the knee jerk, breastfeeding operates via a neural reflex called milk let-down (Figure 1.3). Sensory receptors on the nipple project through afferent fibers directly to the maternal hypothalamus. When an infant begins to suckle, these receptors are activated, and in

turn, inform the hypothalamus to synthesize and release oxytocin (Dawood et al., 1981; Lincoln & Paisley, 1982). Without oxytocin lactation is not possible (Nishimori et al., 1996). Within the hypothalamus, oxytocin is synthesized and secreted from the PVN and SON. It is then released into the bloodstream through the neurohypophysis (posterior pituitary gland), which stimulates the extraction of milk by contracting myoepithelial cells in the mammary gland (Lincoln & Paisley, 1982). This circuit continues until the infant stops suckling.

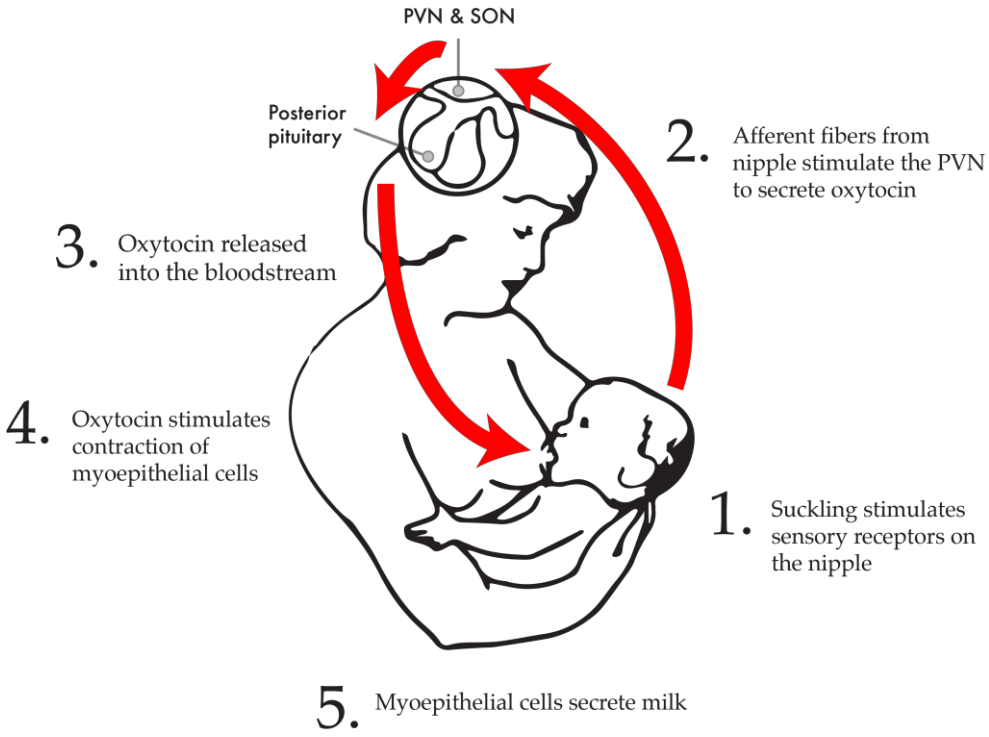

Figure 1.3 Milk let-down is a neural reflex in which the stimulation of the nipple directly activates the release of oxytocin from the brain, and thus, the release of milk to the infant.

The increase in oxytocin during breastfeeding has been well-documented in saliva and plasma of the mother immediately upon suckling (Dawood et al., 1981). A similar rise in oxytocin occurs within the mothers' milk itself (Takeda, Kuwabara, & Mizuno, 1986). Although not directly studied in human infants, breastfeeding has been suggested to increase oxytocin levels in infants directly through breast milk as well as indirectly through caring touch and warmth (Uvnäs-Moberg, 1998). Research with dairy calves and rat pups provides evidence that plasma

oxytocin levels rise as a direct result of suckling (Lupoli, Johansson, Uvnäs-Moberg, & Svennersten-Sjaunja, 2001; Takeda et al., 1986), suggesting a similar effect in other mammalian offspring including human infants. For example, Takeda and colleagues (1986) demonstrated that concentrations of radioactively marked oxytocin injected into rat dams were found in both the plasma and gastric contents of neonates after suckling. Although most hormonal systems operate through negative feedback (e.g. cortisol, testosterone, thyroid hormone), oxytocin is unique in that it utilizes positive feedback mechanisms (Neumann, Russell, & Landgraf, 1993). Therefore, when the brain detects oxytocin, it does not reduce its secretion but instead further stimulates its release.

1.4 A brief history of oxytocin research

1.4.1 Synthesis and receptor distribution

Oxytocin is a highly conserved nanopeptide hormone; it has evolved over 700 million years and homologs are present in taxa from nonvertebrates to mammals (Donaldson & Young, 2009; Garrison et al., 2012; Insel, 1992). As mentioned in the previous section, it is synthesized in the brain within the PVN and SON of the hypothalamus (Laurent, Hindelang, Klein, Stoeckel, & Felix, 1989). It is also synthesized in other regions of the body, including the corpus luteum, uterus, placenta and amnion in women (Chibbar, Miller, & Mitchell, 1993; Lefebvre, Giaid, Bennett, Lariviere, & Zingg, 1992; Lefebvre, Giaid, & Zingg, 1992; Wathes & Swann, 1982) and testis in men (Ang et al., 1991). In the periphery, its main function is to contract smooth muscle tissue and has an important role in mammalian reproduction, most notably during female labor and lactation, but also in male mating behavior including erection and ejaculation (Thackare, Nicholson, & Whittington, 2006). Apart from its peripheral effects, oxytocin is also released directly into the brain from PVN neurons. Brain receptors are located in major limbic regions including the amygdala, nucleus accumbens, and brainstem (Meyer-Lindenberg, Domes, Kirsch,

& Heinrichs, 2011). This allows the hormone to have a direct influence on arousal and behavior (Landgraf & Neumann, 2004).

It is important to note that certain factors can influence the expression and secretion of oxytocin. A substantial body of literature has investigated individual differences on oxytocin pathway genes, such as its receptor gene *OXTR*, and *CD38*. Individual differences in these genes impact plasma oxytocin levels (Feldman et al., 2012) related to differences in socio-emotional functioning (Chen et al., 2011; Sauer, Montag, Reuter, & Kirsch, 2013; Sauer, Montag, Wörner, Kirsch, & Reuter, 2012). For example, *Study 3* (Chapter 5) explores individual differences in the CD38 gene and how genetic variation at this locus impacts emotion processing. CD38 is an ectoenzyme that mediates the release of oxytocin; knockout mice lacking the CD38 gene exhibit dramatically low levels of oxytocin and impaired social behaviors (Jin et al., 2007). Differences within this gene have been linked to autism risk as well as plasma oxytocin levels (Feldman et al., 2012; Lerer et al., 2010; Munesue et al., 2010). Further information on *CD38* can be found in Chapter 5. In the following sections, I will review the literature regarding the influence of oxytocin on behavior.

1.4.2 From maternal care to pair bonds

One of the first observations of oxytocin's impact on behavior can be traced back to the study of maternal care. For example, the infusion of oxytocin has been shown to induce the full range of maternal behaviors in virgin rats, such as licking and grooming pups, building nests, and pup retrieval (Pedersen, Ascher, Monroe, & Prange, 1982). In contrast, the removal of oxytocin function, either through knock-out models, hypothalamic lesions, or with oxytocin antagonists (Insel & Harbaugh, 1989; Rich, deCárdenas, Lee, & Caldwell, 2014; Van Leengoed, Kerker, & Swanson, 1987) has been shown to suppress maternal behaviors in postpartum dams (Insel & Harbaugh, 1989). This shows that oxytocin plays an important role in the instantiation of maternal behaviors.

Oxytocin also plays a strong role in the formation of monogamous pair bonds. The most well-studied species regarding oxytocin and pair bond formation is the prairie vole (*Microtus ochrogaster*). Prairie voles are socially monogamous and exhibit bi-parental care of offspring (Thomas & Birney, 1979; Williams, Catania, & Carter, 1992). The nucleus accumbens of the prairie vole is rich with oxytocin receptors (Insel & Shapiro, 1992; Young, Lim, Gingrich, & Insel, 2001). Injections of an oxytocin receptor antagonist blocks the formation of pair bonds (Young et al., 2001). In female prairie voles, the overexpression of oxytocin receptor in the nucleus accumbens can facilitate pair bond formation in the absence of mating (Keebaugh & Young, 2011; Ross et al., 2009). There are notably different oxytocin receptor distributions in the nucleus accumbens of the monogamous prairie vole when compared to its close relative, the polygamous montane vole (*Microtus montanus*) (Insel & Shapiro, 1992).

Figure 1.4 A prairie vole family. Much of what we know about bonding and oxytocin comes from research in these monogamous animals. Photograph courtesy of Todd Ahern.

The homolog of oxytocin in other taxa also mediates pair bonding behavior. Even *Caenorhabditis elegans*, or roundworms, that lack the oxytocin homolog receptor are not able to

mate successfully (Garrison et al., 2012). Further evidence relating oxytocin to pair bond formation is found in monogamous zebra finches (*Taeniopygia guttata*) (Klatt & Goodson, 2013), teleost fish (*Amatitlania nigrofasciata*) (Oldfield & Hofmann, 2011), and the socially monogamous marmoset (*Callithrix penicillata*) (Freeman, Inoue, Smith, Goodman, & Young, 2014; Smith, Agmo, Birnie, & French, 2010). Work in human adult couples suggests that oxytocin administration is associated with increased levels of pair bonding-related behaviors such as a healthier and more positive communication style during a conflict discussion (Ditzen et al., 2009). Furthermore, higher levels of plasma oxytocin have been found to be positively related to relationship outcomes (Gordon et al., 2008). Another study reported differences in human pair bonding behavior as a function of a single-nucleotide polymorphism (SNP) in the oxytocin receptor (*OXTR* rs7632287), including the partner bonding scale and levels of affection (please see Chapter 2.3 for more information on SNPs). These studies with human adults suggest that oxytocin plays a similar role in bonding behavior in humans as seen in prairie voles (Walum et al., 2012).

1.4.3 Oxytocin and prosocial behaviors

The last decade has seen an explosion of research relating oxytocin and its pathway genes to human prosocial behaviors, such as trust, generosity, as well as emotion recognition and memory (i.e., Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Guastella, Mitchell, & Mathews, 2008; Lukas et al., 2011; Romero, Nagasawa, Mogi, Hasegawa, & Kikusui, 2014; Schulze et al., 2011; Zak, Stanton, & Ahmadi, 2007). In a pioneering study by Kosfeld and colleagues (2005), an economic trust game was played by participants who were either administered intranasal oxytocin or a placebo. The game consisted of an investor and a trustee. The investor was given a choice whether they would like to give money to the trustee. Investors who received oxytocin made the highest possible investment significantly more often than those who received the placebo, and had a higher average investment amount than those who received

placebo. This suggests that oxytocin administration is associated with higher levels of trust. This study also included a critical control condition in which the investor was told that he played with a computer trustee rather than a real human being. In this context, there were no group differences in trusting behavior between the oxytocin and placebo group, suggesting that oxytocin indeed impacted the social elements of trust, and not risk-taking behavior more generally (Kosfeld et al., 2005). The notion that oxytocin administration increases prosocial tendencies in economic games has been replicated across a variety of paradigms, including generosity in the ultimatum game (Zak et al., 2007).

Oxytocin administration has been found to impact the way adults perceive, recognize, and remember emotional stimuli. Individuals administered oxytocin recognize happy facial expressions better, and can do so at low intensities of expression (Marsh, Yu, Pine, & Blair, 2010). Moreover, oxytocin administration reduces the amount of misclassifications of positive expressions as negative expressions (Di Simplicio, Massey-Chase, Cowen, & Harmer, 2009). Happy facial expressions generally indicate one's lack of threat and desire for affiliation (Marsh et al., 2010). Using dynamic morphed stimuli from neutral to anger or happiness, Domes and colleagues (2013) discovered differential eye gaze patterns with oxytocin administration. Oxytocin initially increased eye gaze while both expressions were neutral, however once morphed, eye gaze was significantly strengthened in response to happy faces as compared to angry faces. The notion that oxytocin increases gaze to the eye region is one that has been supported in a number of studies in both autistic and typically developed populations (Andari et al., 2010; Auyeung et al., 2015; Guastella, Mitchell, & Dadds, 2008) (oxytocin and eye gaze is discussed in further depth in *Study 3*, Chapter 5). Oxytocin has also been found to facilitate memory of positive stimuli. Individuals administered oxytocin show increased memory recall specifically to positively-valenced words (Di Simplicio et al., 2009) and remember happy faces better than angry or neutral faces (Guastella, Mitchell, & Mathews, 2008).

This increase in the salience of positive emotions might also be linked to or explained by a reduction of the salience of negative social stimuli (Di Simplicio et al., 2009; Kirsch et al., 2005; Parr, Modi, Siebert, & Young, 2013). There is mounting evidence to implicate the amygdala and connected regions in modulating the effect of oxytocin on facial emotion processing (please see Meyer-Lindenberg et al., 2011 for a review). One current account of oxytocin function, on the basis of a review of available empirical evidence, stipulates that oxytocin increases social approach tendencies while simultaneously decreasing withdrawal tendencies (Kemp & Guastella, 2011) in the service of enhancing social functioning. This theoretical framework can account for seemingly contradictory findings, which on the one hand show that oxytocin administration results in better recognition and retention of positive social stimuli, while on the other hand reducing or dampening the reaction to negative stimuli.

1.4.4 Oxytocin in infants

The aforementioned studies outline the effects of oxytocin administration in adults. However, only very little is known about oxytocin's role in social and emotional functioning in infants. To my knowledge, only two studies have directly investigated potential behavioral effects of oxytocin in infancy. A recent pilot study in human neonates demonstrated that oxytocin levels in the cerebrospinal fluid (CSF) of neonates correlated with infant soothability and sociability at term, 3 months, and 6 months of age as measured by parental self report (Clark et al., 2013). A study in newborn macaque monkeys administered oxytocin through nebulization; that is, oxytocin was inhaled as a mist. Findings from this study revealed that newborns who were nebulized with oxytocin showed greater positive social behaviors, such as increased facial gestures, closer proximity to caregiver, lip smacking, and tongue protrusion (Simpson et al., 2014). Nebulization with oxytocin significantly reduced salivary cortisol levels, which is in congruence with human and animal studies reporting anxiolytic effects of oxytocin (de Oliveira, Zuardi, Graeff, Queiroz, & Crippa, 2012; Yoshida et al., 2009). When conducting infant research,

one must always be careful to use the most non-invasive methods possible. Therefore, the analysis of CSF or plasma oxytocin levels in infants for the purposes of psychological research is rare. Similarly, manipulation of oxytocin levels in human infants through nebulization or intranasal sprays is unethical. Therefore, there is a great need to investigate early exposure to oxytocin in natural situations. Breastfeeding provides an interesting possibility to indirectly and non-invasively investigate the oxytocin system and gives us the opportunity to investigate effects on social functioning in a natural and unobtrusive setting.

Chapter 2. Methodology

Before the presentation of empirical findings, I will briefly summarize three methodologies used in the following experiments. In order to examine infant attentional processes, both neuroscientific and behavioral methods were used: electroencephalography (EEG) and eyetracking. In order to investigate individual differences in attention to emotional information, we focused our analysis on a specific single-nucleotide polymorphism.

2.1 Electroencephalography and event-related potentials

2.1.1 From EEG to ERP

Electroencephalography (EEG) is the most frequently used and established method for studying infant neural activity. EEG is non-invasive and non-painful (de Haan, 2007a). In order to understand how EEG works, one first must understand the brain. Neurons are constantly sending signals to one another, called action potentials. Action potentials involve a large change in the balance of sodium and potassium inside and outside the cell (for a great review, see Barnett & Larkman, 2007). This causes an electric potential due to a change in balance between positive and negative charges. The subsequent electrical potential at a synapse is called an excitatory post-synaptic potential (EPSP). The EPSPs of populations of neurons can be summated into larger, more general electrical potentials, producing waves of electrical activity (Luck, 2005). By placing electrodes on the scalp, this activity can be objectively read via EEG. Many informative markers can be derived from an EEG signal, and the marker chosen depends on the interest of the research question. For example, one can look at specific frequency bands of the signal to assess features such as general attention, sleep and wakefulness patterns, and potential motivational tendencies measured by frontal alpha asymmetry (Davidson, 1984; Fox & Davidson, 1984; Smith,

1938). For the purposes of this thesis, I investigated a specific component of the event-related potential (ERP). While EEG reflects ongoing electrical activity, ERPs reflect changes in activity due to a specific event. One can measure the brain reaction derived from a specific region of the brain time-locked to a specific stimulus. When ERPs are averaged over several trials and multiple subjects, a response waveform is produced (Otten & Rugg, 2005). The waveform produced post-stimulus onset is characterized by positive and negative deflections, or peaks. These peaks have been well-defined and interpreted in both adult and infant literature (de Haan, 2007b; Luck, 2005; Otten & Rugg, 2005). Many components have been defined in terms of brain region (i.e. occipital, frontal), latency, and stimulus modality (i.e. auditory, touch). For the purposes of this thesis I will only discuss one component related to infant visual processing of emotional expressions.

2.1.2 The negative central (Nc) component in infant research

The Nc is one of the most well-studied infant ERP components. It is a negative deflection occurring around 400-800 milliseconds post-stimulus onset, most prominently observed as a negativity at frontal and central electrodes. Under certain conditions, the Nc is comprised of two peaks (Čeponienė et al., 2004; Karrer, Karrer, Bloom, Chaney, & Davis, 1998): an early peak occurring around 300 to 800 ms, and a later peak occurring from around 800 ms. This difference in timing may also map onto distinct cortical sources (Reynolds & Richards, 2005). Classically, the Nc was found to be sensitive to stimulus probability; it was greatest (more negative) in response to less frequent stimuli in oddball paradigms (Courchesne, Ganz, & Norcia, 1981; Karrer et al., 1998). This led researchers to suggest the Nc as a novelty detector. However, the Nc is also more negative in response to well-recognized stimuli. For example, the Nc of six-month-old infants is larger (more negative) in response to their mothers' faces as compared to strangers' faces (de Haan & Nelson, 1997) (see Figure 2.1). That the Nc responds most strongly to less frequent, more novel stimuli (oddball paradigms) as well as more recognized stimuli

suggests instead that the Nc may be a more general indicator of attentional allocation, perhaps marking the salience of a particular stimulus (de Haan, 2007b).

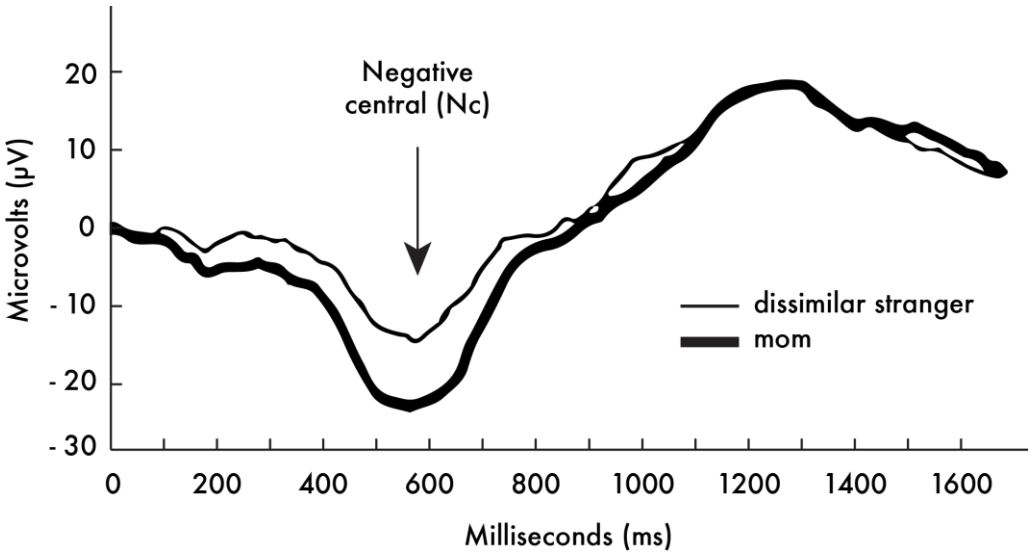

Figure 2.1 Waveform of averaged infant ERPs to images of their own mothers vs. unfamiliar strangers. The Nc component is significantly more negative when viewing images of their own mothers vs. strangers, suggesting an increased attentional allocation. Image adapted from de Haan and Nelson (1997), with permission.

The Nc has been shown to be a sensitive measure of emotion discrimination in infancy. For example, seven-month-old infants display a larger Nc in response to fearful faces as compared to happy faces (Peltola et al., 2009). This suggests that fearful faces are more salient to infants, and thus, more attention is allocated to them. The Nc has also been found to be more negative in response to fearful body expressions as compared to happy expressions (Missana et al., 2014), as well as more negative in response to angry prosody as compared to happy or neutral (Grossmann et al., 2005; Grossmann et al., 2013). These results support the notion that infants develop a strong negativity bias within the first year of life (Vaish, Grossmann, & Woodward, 2008). This theory postulates that infants attribute more salience to stimuli that indicate impending threat, and thus, allocate more attention to negative cues. Apart from marking emotion discrimination in infancy, the Nc is also sensitive to individual differences, such as maternal personality, infant temperament, and genetic variation in neurotransmitter systems (de

Haan, Belsky, Reid, Volein, & Johnson, 2004; Grossmann et al., 2011; Martinos, Matheson, & de Haan, 2012).

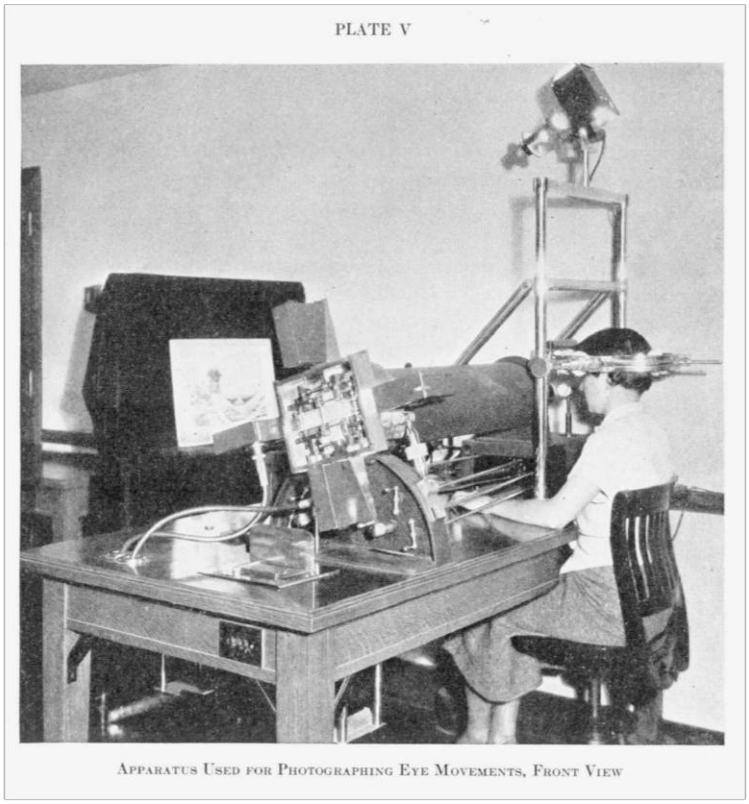

Figure 2.2 Early eyetracking. Photograph of one of the first non-intrusive eye trackers built by Guy Thomas Buswell in Chicago, Illinois, USA (Buswell, 1935). Today, much smaller devices are used. Image reused with permission from University of Chicago Press.

2.2 Tracking eye gaze

By tracking eye gaze, researchers are able to pinpoint which features of a stimulus are most salient to the observer (Duchowski, 2007). The first non-invasive eyetrackers were built in the 1930s (Buswell, 1935) (please see Figure 2.2 for an example of an early apparatus). Using mirrors, researchers reflected light from the foveae of a participant onto photo paper, and an entire looking session could be objectively recorded. Today, eyetracking technology has transformed into much more user-friendly techniques. The eyetracker methodology used in the current thesis

uses infrared light. After calibration, the eyetracker is able to reference reflected light from the fovea and thus, track the looking behavior of a participant of any age (Holmqvist et al., 2011).

Eyetracking is useful for infant research because it is non-invasive and does not require many trials, meaning less time required for the experiment. Infants sit on a parent's lap and are able to freely view stimuli presented to them on a computer monitor. We are then able to make inferences regarding infant visual attention (Gredeback, Johnson, & von Hofsten, 2009). Several informative variables can be measured with eyetracking. For example, sympathetic nervous system activity can be assessed through pupil dilation in response to certain stimuli, which can be a marker of arousal (Bradley, Miccoli, Escrig, & Lang, 2008). As I was interested in the allocation of attention in the current thesis, I investigated infants' total looking duration to the eyes of emotional faces (see *Study 3,* Chapter 5).

2.3 The investigation of single-nucleotide polymorphisms (SNPs)

In *Study 3* (Chapter 5), I investigated whether genetic differences may interact with experience to impact emotion perception. First, it is necessary to give a brief introduction of DNA. DNA is a long, complex molecule divided into chromosome pairs (one set from the mother, one set from the father). DNA is comprised of nucleotides arranged in base pairs. These nucleotide bases are: adenine (A), thymine (T), guanine (G), and cytosine (C). The human genome consists of roughly three billion base pairs. In total, these base pairs comprise about 25,000 genes, which can be decoded into proteins (Plomin, DeFries, Knopik, & Neiderhiser, 2013). Sequences of three base pairs are called codons, which are translated into amino acids. For example, the hormone oxytocin consists of nine amino acids. The great majority of amino acids can be coded by more than one codon. For example, the amino acid glutamine can either be transcribed and translated from the sequence GTT or GTC. Consequently, for many of our

genes, polymorphisms in particular single nucleotide bases (a single locus) can be found. These are called single-nucleotide polymorphisms (SNPs) (Syvanen, 2001) (Figure 2.3).

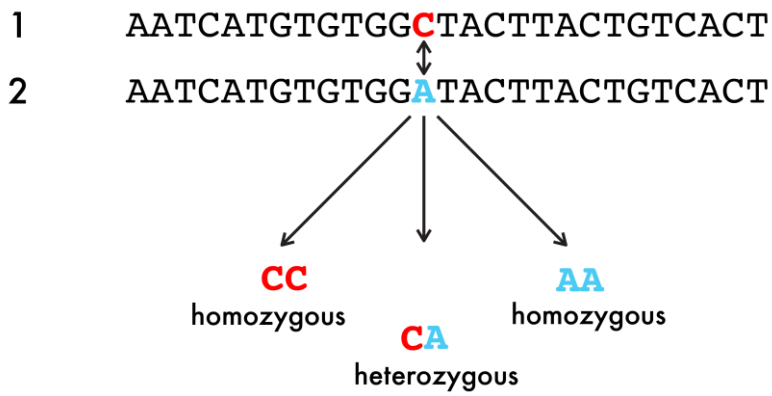

Figure 2.3 Single-nucleotide polymorphisms (SNPs). On certain sequences of DNA, one allele locus can vary between individuals and can be either one of two nucleotide bases. For example, in the schematic above, the highlighted locus can be either cytosine (C) or adenine (A). Because we are given DNA from both our mothers and fathers, one can either have a homozygous (CC or AA) or heterozygous (CA) genotype for a particular SNP.

Most SNPs are synonymous, meaning that the change in base pair does not alter the translation of the specific amino acid (as seen above with glutamine). However, synonymous SNPs may display subtle effects by changing the rate that mRNA is translated into proteins. Even more interesting is that SNPs found on non-coded regions of the genome can impact phenotypes (Plomin et al., 2013). Only about 2% of the genome consists of sequences which are translated into amino acids, called exons. The great majority of DNA consists of introns, located between exons. Introns are still transcribed into mRNA, but are spliced out before translation into amino acids occurs. For example, in *Study 3*, I will discuss a common SNP on the *CD38* gene. This SNP is located on the intron of the gene, so it is not involved in the translation of the base pairs into amino acids. However, differences within this SNP have been found to impact both biological and behavioral phenotypes related to the oxytocin system and social functioning (Feldman et al., 2012; Sauer et al., 2013; Sauer et al., 2012). Thus, it is likely to be involved in regulating the transcription rate of its respective gene or that of other genes.

Part II. Empirical investigations

Research Questions

As summarized above, a substantial body of literature has highlighted the influential role breastfeeding plays in both mothers and infants. Notably, breastfeeding duration has been associated with a variety of beneficial cognitive and neural outcomes in offspring, and there is increasing evidence that these effects may persist into adulthood. Despite its recognized influence on brain and cognitive development, as well as its stress-reducing and mood-elevating properties in mothers, there is very little research concerning potential social outcomes of breastfeeding. This is surprising, not only due to its strong relationship with oxytocin, a hormone known to influence social behavior, but also due to the nature of breastfeeding. It is a dynamic, social behavior between two individuals. One integral component of optimal social functioning is the ability to detect and distinguish between others' emotional expressions. Thus, in the following three experiments, I investigated whether breastfeeding experience and variation within the oxytocin system can impact social responding. I explored emotion perception as a crucial aspect of human social responding, and considered this skill in both mothers and infants. Because oxytocin has been found to increase the salience of positive social cues and reduce that of negative social cues, I hypothesized that breastfeeding would act in a similar fashion: that is, longer durations of exclusive breastfeeding experience might increase attentional allocation to positive emotional expressions and decrease attention toward negative emotional expressions.

Study 1. Does exclusive breastfeeding duration impact emotion recognition in mothers? In this study, I investigated whether breastfeeding experience impacts emotion recognition in healthy mothers of five- to seven-month-old infants. Mothers were administered a dynamic emotion recognition task in which they were asked to recognize emotional facial expressions as quickly and accurately as they could. I hypothesized that, due to elevated oxytocin levels during breastfeeding, mothers with longer exclusive breastfeeding experience would have an increased sensitivity to positive

expressions and a decreased sensitivity to negative (threatening) expressions as evidenced by their reaction times.

Study 2. Does exclusive breastfeeding duration impact emotion perception in infants? In this study, I investigated whether breastfeeding experience impacts infant neural responses to emotional body expressions. Eight-month-old infants were presented with happy and fearful static body expressions while EEG recorded neural responses. In line with *Study 1*, I hypothesized that infants with a longer duration of exclusive breastfeeding would have a stronger neural response to the happy body expressions than infants with a shorter exclusive breastfeeding duration.

Study 3. Might exclusive breastfeeding duration and genetic differences within the oxytocin system interact to impact infant attention to emotional expressions? In this study, I investigated infant looking preferences to the eye region of facial emotional expressions using an eyetracking paradigm. Genetic information regarding a single-nucleotide polymorphism (SNP) on the CD38 gene (rs3796863) was collected and analyzed. First, I hypothesized that infants with a longer duration of exclusive breastfeeding would display a greater preference towards happy eyes than those with a lower duration of exclusive breastfeeding. Second, I hypothesized that the impact of breastfeeding on looking preferences might interact with one's own capacity to release oxytocin (as measured through *CD38* rs3796863 genotype).

Chapter 3.
Study 1: Breastfeeding experience differentially impacts recognition of happiness and anger in mothers[2]

Kathleen M. Krol[a], Sunjeev Kamboj[b], H. Valerie Curran[b], & Tobias Grossmann[a,c]

[a]Early Social Development Group, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; [b]Clinical Psychopharmacology Unit, Research Department of Clinical, Educational and Health Psychology, University College London, London, UK; [c]Department of Psychology, University of Virginia, Charlottesville, USA

Abstract. Breastfeeding is a dynamic biological and social process based on hormonal regulation involving oxytocin. While there is much work on the role of breastfeeding in infant development and on the role of oxytocin in socio-emotional functioning in adults, little is known about how breastfeeding impacts emotion perception during motherhood. We therefore examined whether breastfeeding influences emotion recognition in mothers. Using a dynamic emotion recognition task, we found that longer durations of exclusive breastfeeding were associated with faster recognition of happiness, providing evidence for a facilitation of processing positive facial expressions. In addition, we found that greater amounts of breastfed meals per day were associated with slower recognition of anger. Our findings are in line with current views of oxytocin function and support accounts that view maternal behavior as tuned to prosocial

[2] Krol, K. M., Kamboj, S. K., Curran, H. V., & Grossmann, T. (2014). Breastfeeding experience differentially impacts recognition of happiness and anger in mothers. *Scientific Reports, 4*, 7006.

responsiveness, by showing that vital elements of maternal care can facilitate the rapid responding to affiliative stimuli by reducing importance of threatening stimuli.

3.1 Introduction

Breastfeeding is a dynamic process that is characterized by physical, hormonal, and psychosocial effects in both mothers and infants (Deoni et al., 2013; Heinrichs et al., 2002; Raju, 2011). In particular, exclusive breastfeeding duration has been shown to play a vital role in bonding between mother and child through the promotion of positive affect and sensitive maternal behaviors (Brandt et al., 1998; Kim et al., 2011; Zetterström, 1999). Critically, exclusively breastfeeding mothers report lower stress, negative moods, and anxiety than formula-feeders (Groër, 2005), and this anxiolytic impact is further evidenced through stronger cardiac vagal tone modulation, reduced heart rate reactivity (Mezzacappa et al., 2005), blood pressure (Hahn-Holbrook et al., 2011) and reduced hypothalamic-pituitary-adrenal axis activity to psychosocial stress (Heinrichs et al., 2002). Reduced blood pressure has also been associated with the percentage of meals still breastfed (Hahn-Holbrook et al., 2011). At the hormonal level, lactation requires oxytocin, a neurohormone synthesized in the paraventricular and supraoptic nuclei of the hypothalamus. Milk let-down is a process in which infant suckling induces release of oxytocin into the bloodstream through the neurohypophysis, stimulating the extraction of milk through contraction of myoepithelial cells in the mammary gland (Mezzacappa et al., 2005). Oxytocin is simultaneously released into the brain via projections from the paraventricular nucleus, allowing for the hormone to have a direct influence on behavior (Landgraf & Neumann, 2004). The increase in peripheral oxytocin during breastfeeding has been well-documented in saliva and plasma of mothers during the feeding process (Dawood et al., 1981). Furthermore, breastfeeding mothers have higher levels of oxytocin in general as compared to formula-feeders (Grewen, Davenport, & Light, 2010), making increased levels of oxytocin a characteristic trait of lactating mothers.

Oxytocin is well-known for its peripheral role in the female reproductive system; however research over the last two decades has demonstrated that it also plays a critical role in affiliative

and prosocial behavior (Heinrichs, von Dawans, & Domes, 2009; Insel, 1992). Intranasal oxytocin administration increases the salience of eyes in faces, enhances trust, generosity, social memory, and emotion recognition in humans (Guastella, Mitchell, & Dadds, 2008; Guastella, Mitchell, & Mathews, 2008; Kosfeld et al., 2005; Zak et al., 2007). There is accumulating evidence that oxytocin might foster affiliative behavior through both the perceptual enhancement of positive emotional cues and the perceptual reduction of negative, threatening cues (Di Simplicio et al., 2009; Domes, Steiner, et al., 2013; Kirsch et al., 2005; Marsh et al., 2010; Parr et al., 2013; Schulze et al., 2011). This may be due to a mechanism in which oxytocin promotes the facilitation of approach-behaviors and reduces the tendency of withdrawal-behaviors (Kemp & Guastella, 2011). Neuroimaging studies suggest this might be due to an attenuation of the amygdala as well as a reduction in functional coupling to regions of the brainstem in response to threatening scenes (Gamer, Zurowski, & Buchel, 2010; Kirsch et al., 2005; Petrovic, Kalisch, Singer, & Dolan, 2008).

As breastfeeding mothers exhibit an extended release of oxytocin (Dawood et al., 1981), the current study investigated whether breastfeeding experience promotes enhanced socio-emotional perception. Using a dynamic facial expression recognition task (Platt, Kamboj, Morgan, & Curran, 2010), we explored whether breastfeeding behavior, as indexed through both exclusive breastfeeding duration and current breastfeeding exposure (percentage of infant's diet still breastfed), is associated with individual differences in emotion processing in mothers. Based on theoretical accounts that assign a primary role to breastfeeding and oxytocin in maternal sensitivity (Kim et al., 2011) and prior empirical work relying on intranasal administration of oxytocin (Di Simplicio et al., 2009; Domes, Steiner, et al., 2013; Guastella, Mitchell, & Mathews, 2008; Kirsch et al., 2005; Marsh et al., 2010; Parr et al., 2013; Schulze et al., 2011), we hypothesized that a greater exposure to breastfeeding would be associated with greater sensitivity to happy expressions while reducing sensitivity to expressions of threat (especially anger). As the majority of the aforementioned breastfeeding studies examined outcomes of exclusive

breastfeeding duration, we used this index as our main variable of interest. Furthermore, focusing our investigation on differences in exclusive breastfeeding allowed us to examine variation within a group of breastfeeding mothers rather than coarsely contrasting formula feeding mothers to breastfeeding mothers.

Table 3.1 Infant and maternal characteristics.

	Descriptives		Pearson's *r*	
	Range	*M*(*SE*)	*EBF*	*%BFM*
EBF duration (days)	91–213	159.21 (3.67)		.662***
Breastfed meals (%)	0–100	63.75 (4.23)	.662***	
Maternal				
Age (years)	22–42	32.33 (0.53)	.135	.201
Education (years)	10–26	15.28 (0.42)	−.016	.340**
Other children (count)	0–2	0.53 (0.07)	−.131	−.035
PANAS				
Positive affect	19–38	14.75 (0.31)	−.174	−.128
Negative affect	21–39	30.48 (0.81)	.228	.201
IRI				
Empathic concern	10–20	14.75 (0.31)	.063	−.019
Perspective taking	7–20	13.49 (0.39)	.009	.039
Personal distress	5–17	11.36 (0.38)	−.116	.029
Infant				
Age (days)	166–226	195.79 (2.45)	.060	−.346**
IBQ-R				
Smiling & laughter	2.50–5.89	4.23 (0.11)	−.185	−.122

Note: EBF = Exclusive breastfeeding duration, PANAS = Positive and Negative Affect Schedule, IRI = Interpersonal Reactivity Index, IBQ-R = Revised Infant Behaviour Questionnaire;
**p < .01,
***p < .001.

3.2 Results

3.2.1 Breastfeeding characteristics

Out of 62 mothers included in our sample, the great majority (53 mothers: 85.5%), were still breastfeeding their infants on a daily basis. All mothers breastfed exclusively for at least 3 months (*range* =91-213 days; *M* = 159.21, *SD* = 28.91) (Table 3.1). Using correlations, we examined the possibility that empathic concern, positive or negative affect, infant positive affect

(smiling and laughter), maternal education, or parity impact breastfeeding behavior. The duration of exclusive breastfeeding did not relate to any of these measures (all *p*-values > .18). However, the percentage of breastfed meals per day related to maternal education ($r(62) = .32$, $p = .007$), infant age ($r(62) = -.346$, $p = .006$), and exclusive breastfeeding duration ($r(62) = .662$, $p < .001$). Therefore years of maternal education and infant age were included as regression predictors in all analyses.

3.2.2 DEER-T performance

In our analysis, we tested the hypothesis that greater exposure to breastfeeding would be associated with greater sensitivity to happy expressions and reduced sensitivity to threatening expressions (especially anger). As a first step, correlations were performed in order to investigate relationships between breastfeeding behavior and DEER-T performance. All significant correlations were subsequently entered into multiple regression analyses. This initial correlation analysis mainly served a protective function, namely, to identify potentially confounding factors and include those in the regression model designed to test the main hypothesis. Exclusive breastfeeding duration correlated only with reaction time (RT) to happiness ($r(62) = -.268$, $p = .035$). The percentage of breastfed meals correlated significantly with false alarms (FAs) to anger and fear ($r(62) = -.263$, $p = .039$ and $r(62) = -.348$, $p = .006$, respectively) as well as RTs to anger and fear ($r(62) = .287$, $p = .024$ and $r(62) = -.325$, $p = .010$, respectively). With this information, forced-entry multiple regressions were conducted analyzing FAs (anger, fear) and RTs (anger, fear, happiness). Exclusive breastfeeding duration, percentage of breastfed meals, infant age, and maternal education were entered as predictors. No predictors remained significant for FAs, suggesting that after including variance from other factors, FAs to anger and fear no longer related to the percentage of breastfed meals (all β-values < -.187, *p*-values > .240). Additionally, the percentage of breastfed meals no longer predicted the RT to fear ($\beta < -.200$, $p > .343$).

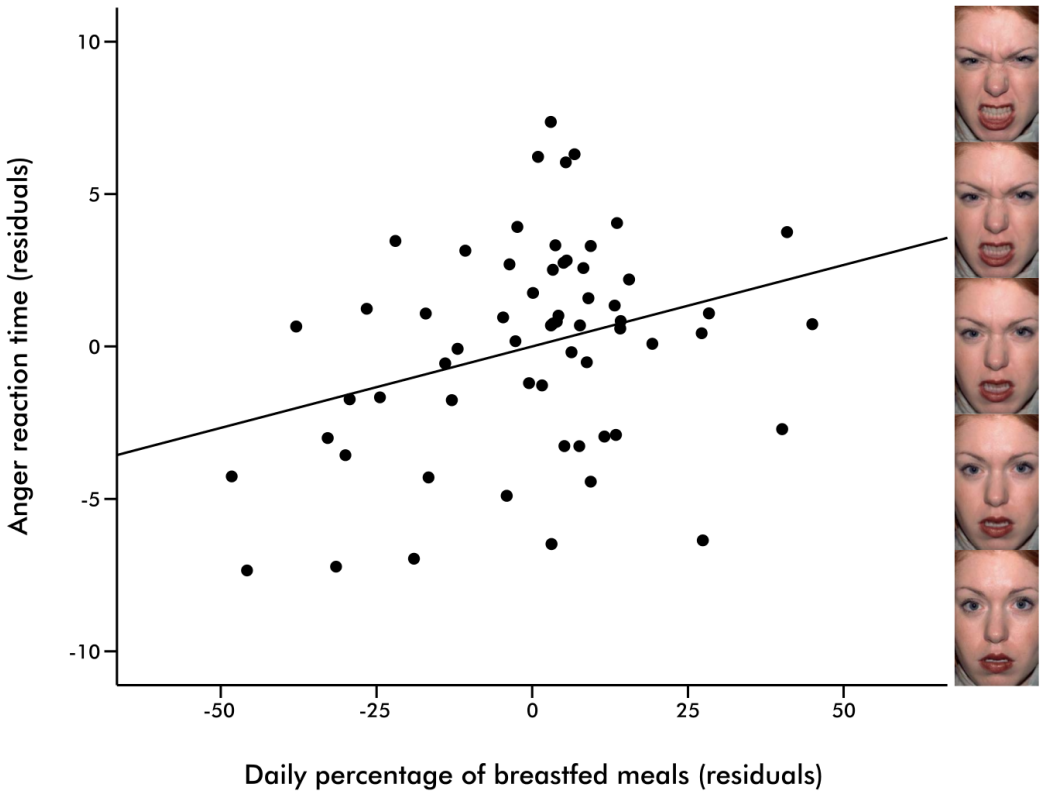

Figure 3.1 Current breastfeeding behavior and anger recognition. Partial regression plot in which the percentage of currently breastfed meals predicts RT to anger, $\beta = .53$, $p = .014$. RT increases with a larger percentage of breastfed meals. Panel on the right depicts snapshots at five time points during a dynamic morph from neutral to anger. Note: Photographs are from the NimStim Face Stimulus Set. Development of stimuli was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development. This set is available to researchers at www.macbrain.org/resources.htm.

Table 3.2 Summary of multiple regression analyses for variables predicting reaction time to anger, $R^2 = .161$, $N = 62$.

Variable	*B*	*SE B*	β	*t*	*p*-value
(Constant)	42.23	7.21		5.86	.000
EBF (in days)	−.05	.03	−.30	−1.60	.115
Breastfed meals (%)	.07	.03	.53	2.54	.014
Infant age	.03	.03	.13	.86	.392
Maternal education	−.01	.18	−.01	−.05	.961

A significant impact of breastfeeding exposure remained for RTs to anger and happiness. The percentage of breastfed meals was the strongest predictor for the reaction time to anger (Table 3.2, Figure 3.1), in which the higher the percentage, the slower the RT to anger. Exclusive breastfeeding duration was the strongest predictor for the speed of processing happy facial

expressions (Table 3.3, Figure 3.2). Specifically, our analysis revealed an acceleratory effect of exclusive breastfeeding on the RT to happiness, providing evidence for a facilitation of processing positive emotional signals. Note that all *p*-values reported here are uncorrected and would not survive a conservative Bonferroni correction.

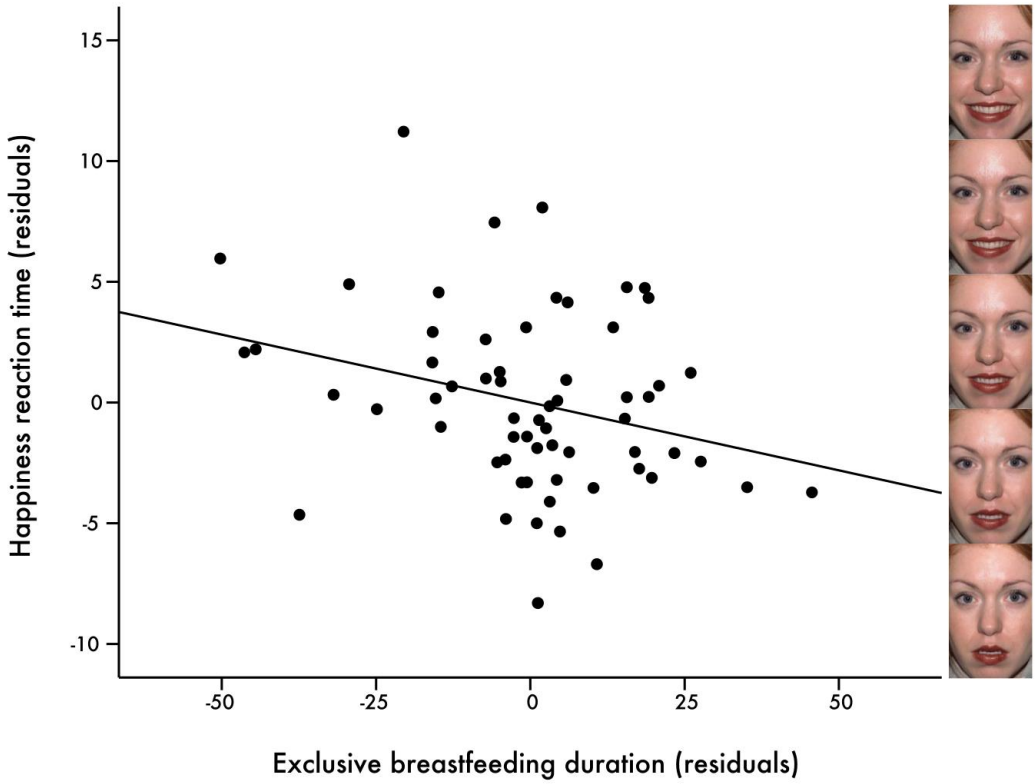

Figure 3.2 Exclusive breastfeeding duration and happiness recognition. Partial regression plot in which EBF significantly predicts RT to happiness, β = -.42, p = .029. RT decreases with a longer EBF duration. Panel on the right depicts snapshots at five time points during a dynamic morph from neutral to happiness. Note: Photographs are from the NimStim Face Stimulus Set. Development of stimuli was overseen by Nim Tottenham and supported by the John D. and Catherine T. MacArthur Foundation Research Network on Early Experience and Brain Development. This set is available to researchers at www.macbrain.org/resources.htm.

Table 3.3 Summary of multiple regression analyses for variables predicting reaction time to happiness, R^2 = .142, N = 62.

Model	*B*	*SE B*	β	*t*	*p*-value
(Constant)	54.41	6.44		8.44	.000
EBF (in days)	−.06	.03	−.42	−2.25	.029
Breastfed meals (%)	.03	.02	.24	1.17	.246
Infant age	−.03	.03	−.15	−1.06	.293
Maternal education	−.19	.16	−.16	−1.15	.254

3.3 Discussion

In the current study, we examined whether breastfeeding exposure affects emotion recognition in mothers. Using a dynamic emotional face recognition task, we found that faster recognition of happiness was associated with longer exclusive breastfeeding duration in mothers. Furthermore, a greater percentage of currently breastfed meals was associated with slower reaction times toward anger. Thus, our data show that breastfeeding behavior is related to an increased sensitivity to positive emotional cues and a decreased sensitivity to negative, threat-related cues. This pattern of results is in line with existing accounts of oxytocin function, according to which oxytocin is considered to enhance approach tendencies and inhibit withdrawal tendencies in an effort to facilitate prosocial behavior (Kemp & Guastella, 2011). Moreover, these findings generally support the notion that breastfeeding behavior might be associated with what has been referred to as a positivity bias in emotion perception (Peeters, 1971). Critically, these findings cannot be explained by other variables such as maternal education, maternal mood, parity, or infant temperament, suggesting that variation in breastfeeding itself accounts for differences in emotion processing in motherhood.

Our findings support the view according to which maternal behavior is tuned to prosocial responsiveness and bonding, and its vital elements can facilitate the rapid and accurate responding to affiliative cues (Feldman, 2012c). In this context, it is important to note that the mothers in the current study showed this bias in their emotion recognition in response to unfamiliar persons. This suggests that the breastfeeding behavior, while certainly having specific effects on the relationship between infant and mother (Kim et al., 2011; Raju, 2011; Zetterström, 1999), is also linked to more general emotion processing differences that can be observed in response to unfamiliar persons. To find that specific aspects of maternal behavior can have general effects on prosocial responsiveness is in line with accounts that assign an important role to maternal behaviors in the evolution of human prosociality (Hrdy, 2009).

Oxytocin release is required for the process of milk let-down (Lincoln & Paisley, 1982), and its heightened release during breastfeeding has been well-documented (Dawood et al., 1981). Our results are in agreement with a host of studies showing that exogenous oxytocin administration impacts the perception of emotional expressions (Di Simplicio et al., 2009; Domes, Steiner, et al., 2013; Guastella, Mitchell, & Mathews, 2008; Kirsch et al., 2005; Marsh et al., 2010; Parr et al., 2013; Schulze et al., 2011). Specifically, the current findings are in line with oxytocin administration studies in that these studies also find an enhanced responding to positive emotional cues (related to approach tendencies) and a reduced responding to negative, threat-related cues (related to withdrawal tendencies) (Kemp & Guastella, 2011). It is thus conceivable that our current findings are related to the endogenous release of oxytocin during breastfeeding. However, it must be stressed that we did not directly measure oxytocin levels in our sample of mothers. Therefore, until directly tested, increased oxytocin release must only be considered a potential pathway (among others) that can account for our current results.

The bias in emotion recognition observed in the current study may also be related to the noted anxiolytic effect of breastfeeding (Mezzacappa et al., 2005). Indeed, there is evidence that anxiolytic drugs such as diazepam increase the sensitivity towards happiness (Murphy, Downham, Cowen, & Harmer, 2008) while decreasing recognition of fear and anger in facial expressions (Zangara, Blair, & Curran, 2002). This is also seen during administration of selective serotonin reuptake inhibitors (SSRIs), which exert anxiolytic effects (Harmer, Shelley, Cowen, & Goodwin, 2004). Oxytocin itself exerts anxiolytic effects in both mice (Yoshida et al., 2009) and humans (de Oliveira et al., 2012). It has close ties to the serotonergic system, and facilitates serotonin secretion in the raphe nucleus (Yoshida et al., 2009). Moreover, breastfeeding involves skin-to-skin contact and pleasant touch, which has been found to influence emotion regulation, attention, joint engagement, and pain analgesia (Feldman, Rosenthal, & Eidelman, 2014; Gray, Watt, & Blass, 2000). In this context, it should be emphasized that breastfeeding is a highly complex and dynamic biological and psychological process, and more systematic research is

required to understand what exactly underpins the effects observed in the current study. Therefore, we would like to stress that our study is only a first step. Future work using a systematic assessment of hormonal, genetic, neural, and behavioral variables in mothers will be vital in order to achieve a more detailed and possibly more mechanistic understanding of the effects of breastfeeding on socio-emotional processing.

Another important issue raised by the current data is the question why exclusive breastfeeding duration is associated with recognition of happy faces, while current breastfeeding exposure relates to differences in anger recognition. One possibility is that there might be differences in emotion perception due to chronic (exclusive breastfeeding) versus acute (current breastfeeding) effects of breastfeeding exposure. More specifically, while long-term breastfeeding might lead to changes in receptor affinities or distributions (perhaps in the oxytocinergic system), more acute effects of breastfeeding might have the greatest impact on arousal systems important for threat detection, such as the amygdala and brainstem regions. To our knowledge, there is currently no research that has directly assessed this issue by comparing between current breastfeeding behavior and exclusive breastfeeding duration. However, animal research confirms that chronic oxytocin exposure is capable of changing oxytocin receptor distributions (Insel, Winslow, & Witt, 1992), and most imaging studies indicate that acute oxytocin administration seems to primarily target reactivity of the amygdala (Domes, Heinrichs, Glascher, et al., 2007; Gamer et al., 2010; Kirsch et al., 2005; Petrovic et al., 2008). In future work it will be important to distinguish between long-term and short-term effects of breastfeeding.

Relatedly, it is unclear whether the positive and negative biases found in the current sample persist after the cessation of breastfeeding. Our study was not designed to directly address this question as only nine mothers in the current sample had ceased breastfeeding. However, an exploratory correlation analysis conducted with the nine mothers that had stopped breastfeeding revealed an association between exclusive breastfeeding duration and reaction time to happy expressions ($r(9) = -.624$, $p = .037$, one-tailed), similar to the mothers that were still breastfeeding.

This suggests that some of the effects may persist after the cessation of breastfeeding, but clearly more work is needed to directly test this possibility. Finally, one limitation of the current study is that, as stated in the results section, we did not control for multiple comparisons and only report uncorrected results. However, it is important to note that the current study tested and provides support for a specific a priori hypothesis formulated on the basis of previous work (Di Simplicio et al., 2009; Domes, Steiner, et al., 2013; Kirsch et al., 2005; Marsh et al., 2010; Parr et al., 2013; Schulze et al., 2011).

In conclusion, our results provide evidence that mothers who exclusively breastfeed for longer durations show an increased sensitivity to positive facial expressions. Moreover, mothers with an increased percentage of daily breastfed meals show a decreased sensitivity to anger. The finding that such emotional biases occur in the context of the psychological and biological processes associated with breastfeeding is testament for a need to better understand the impact that maternal behaviors in general and breastfeeding in particular have on socio-emotional functioning during motherhood.

3.4 Methods

3.4.1 Participants

62 healthy women of European descent (M_{age} = 32.33 years, SD = 4.17) participated in the study. All were mothers of five- to seven-month-old infants (M_{age} = 6.43 months, SD = .63). All mothers were on maternity leave up to the point of testing. All gave informed consent and were compensated with travel money and a toy for their infant. Procedures were approved by the ethics committee of the Leipzig University Medical School and were conducted in accordance with the Declaration of Helsinki.

3.4.2 Questionnaires

We obtained information concerning maternal characteristics (age, years of education, number and age of other children, immigration history, highest academic/professional qualification) as well as the following information concerning breastfeeding: the duration a mother exclusively breastfed her child, at what age the child was introduced to other foods, and/or at what age the mother stopped breastfeeding, through an in-house developed questionnaire. As part of this questionnaire, a table was provided in which mothers could describe a feeding schedule over the course of a typical day. Based on this information, the frequency and percentage of breastfed meals per day was calculated. Durations provided in months were converted into days. If mothers were still exclusively breastfeeding, infants' age on testing day was used as exclusive breastfeeding duration to be as specific as possible. In addition, the Infant Behavior Questionnaire in its revised form (IBQ-R), the Interpersonal Reactivity Index (IRI), and the Positive and Negative Affect Schedule (PANAS) (Davis, 1983b; Gartstein & Rothbart, 2003; Watson, Clark, & Tellegen, 1988) were administered in order to investigate potential influences of infant temperament, maternal affect, and maternal empathy on both emotion recognition performance and breastfeeding behavior. The IBQ-R is commonly used instrument to assess infant temperament through parental report. It includes subscales assessing infant expression of smiling and laughter, approach tendency, perceptual sensitivity, cuddliness, fear, rate of recovery from distress, and many others. Gartstein and Rothbart (2003) provide evidence supporting the instrument's high reliability and validity. The IRI (Davis, 1983b) uses a multi-dimensional approach to assess empathy in adults. It assesses the tendency one has to take the point of view of others (PT), the tendency to experience feelings of sympathy and compassion for those less fortunate (EC), the tendency to experience feelings of distress in response to discomfort in others (PD), and the tendency to transpose oneself into fictional situations such as novels or movies (FS). The IRI has also been reported to be a reliable and valid instrument that assesses empathy in adults (Davis, 1983b). The PANAS measures affect on two

subscales in adults: positive affect (i.e., enthusiasm and alertness), and negative affect (i.e., aversive mood states and general distress). Watson and colleagues (1988) report high reliability and validity of the PANAS.

3.4.3 The Dynamic Emotional Expression Recognition Task (DEER-T)

Emotion recognition was measured using the DEER-T, which has been used to study emotion recognition in various populations and is described in detail elsewhere (Platt et al., 2010). Neutral color photographs of twelve Caucasian actors (six women) were morphed to create six dynamic expressions: anger, happiness, fear, sadness, disgust, and neutrality. Images were taken from the NimStim set, which is freely available to the scientific community at http://www.macbrain.org/resources.htm (Tottenham et al., 2009). Stimuli were displayed as dynamically morphing from neutral into a full-blown emotion over the course of 3000 milliseconds. Six labeled response keys corresponded to the emotions. To reduce memory load, an illustration of the keys in relation to finger positions was displayed on the monitor below the presented stimuli. Participants were instructed to press the key of the corresponding emotion as quickly and accurately as they could. Stimuli were presented on a 13-inch laptop.

Performance on the DEER-T was assessed using reaction time (RT), hits, and false alarms (FAs). RTs were analyzed for correct answers only. Hits refer to the correct response frequency for a particular emotion, while FAs refer to the frequency a participant responded with an emotion when it was incorrect. RTs were square-root transformed to correct for positive skew. Data was analyzed with IBM SPSS Statistics (Version 19).

Acknowledgments

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Chapter 4.
Study 2: Duration of exclusive breastfeeding is associated with differences in infants' brain responses to emotional body expressions[3]

Kathleen M. Krol[a], Purva Rajhans[a], Manuela Missana[a], & Tobias Grossmann[a,b]

[a]Early Social Development Group, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; [b]Department of Psychology, University of Virginia, Charlottesville, USA

Abstract. Much research has recognized the general importance of maternal behavior in the early development and programming of the mammalian offspring's brain. Exclusive breastfeeding duration, the amount of time in which breastfed meals are the only source of sustenance, plays a prominent role in promoting healthy brain and cognitive development in human children. However, surprisingly little is known about the influence of breastfeeding on social and emotional development in infancy. In the current study, we examined whether and how the duration of exclusive breastfeeding impacts the neural processing of emotional signals by measuring electro-cortical responses to body expressions in eight-month-old infants. Our analyses revealed that infants with high exclusive breastfeeding experience show a significantly greater neural sensitivity to happy body expressions than those with low exclusive breastfeeding

[3] Krol, K. M., Rajhans, P., Missana, M., & Grossmann, T. (2015). Duration of exclusive breastfeeding is associated with differences in infants' brain responses to emotional body expressions. *Frontiers in Behavioral Neuroscience, 8*, 459.

experience. Moreover, regression analyses revealed that the neural bias toward happiness or fearfulness differs as a function of the duration of exclusive breastfeeding. Specifically, longer breastfeeding duration is associated with a happy bias, whereas shorter breastfeeding duration is associated with a fear bias. These findings suggest that breastfeeding experience can shape the way in which infants respond to emotional signals.

4.1 Introduction

4.1.1 Emotion discrimination in infancy

The ability to perceive and distinguish between the emotional states of others is a crucial social skill that helps us predict others' actions and guide our own behavior during social interactions (Frith, 2009). Emotional communication is inherently multisensory, as information about another person's emotional state can be gleaned from various channels (Heberlein & Atkinson, 2009). Most research has focused on the perception of emotions from facial and vocal expressions (Belin, Campanella, & Ethofer, 2013), even though body expressions may be the most evolutionarily preserved and immediate means of conveying emotional information (Aviezer, Trope, & Todorov, 2012; de Gelder, 2006). Adults are readily able to detect and recognize various emotions from body expressions (Atkinson, 2013; de Gelder, 2009). This ability to recognize emotions from body expressions relies on specific brain processes localized principally in the right hemisphere, including superior temporal, somatosensory and premotor cortices (Atkinson, 2013; de Gelder, 2006; Grèzes, Pichon, & de Gelder, 2007; Heberlein, Adolphs, Tranel, & Damasio, 2004; Heberlein & Saxe, 2005).

In development, the ability to respond to emotional information emerges during the first year of life, during which time infants become sensitive to various facial, vocal, and body expressions (Grossmann, 2013; Missana et al., 2015; Missana et al., 2014). There is behavioral and neural evidence to suggest that infants develop the ability to detect and discriminate between others' positive and negative emotional expressions, tending to view the negative expressions as more salient (Vaish et al., 2008). For example, seven-month-old infants but not five-month-old infants show longer looking times to fearful faces than to happy faces and an enhanced Negative central (Nc) component in their event-related brain potential (ERP), indexing greater allocation of processing and attentional resources to fearful expressions (Kotsoni et al., 2001; Nelson & de Haan, 1996; Peltola et al., 2009). Similarly, seven-month-olds show an enhanced neural sensitivity

to angry voices when compared to happy and neutral voices (Grossmann, Oberecker, Koch, & Friederici, 2010; Grossmann et al., 2005). Only recently has research begun to investigate the development of emotional body processing in infancy. Similar to what has been shown with facial and vocal stimuli, eight-month-old infants show an enhanced neural sensitivity to fearful relative to happy body expressions in both dynamic (Missana et al., 2015) and static stimuli (Missana et al., 2014).

Given this well-mapped emergence of the neural sensitivity to emotional information, it appears particularly important to understand which factors might contribute to individual differences during this early stage of development. To gain a better understanding of this might help inform theories about how certain biases in emotional processing come about. Such biases are thought to have long-term beneficial or detrimental effects on the well-being and development of an individual (i.e. negativity versus positivity bias) (Fox, 2012; Roiser, Elliott, & Sahakian, 2012; Sharot, 2011; Vaish et al., 2008). In prior infant research, the focus of individual differences in the neural processing of emotions has mainly been on intrinsic factors such as genetic variation in neurotransmitter systems (Grossmann et al., 2011; Grossmann et al., 2013) and variation in temperament with respect to emotion regulation and expression (de Haan et al., 2004; Grossmann et al., 2011; Grossmann et al., 2013; Martinos et al., 2012). However, little is known about how certain experiential factors are linked to emotion perception and its neural underpinnings in infancy.

4.1.2 Exclusive breastfeeding, development, and oxytocin

Prior research has recognized the general importance of maternal behavior and care in the early development and epigenetic programming of the mammalian offspring's brain (Cushing & Kramer, 2005; Masís-Calvo, Sequeira-Cordero, Mora-Gallegos, & Fornaguera-Trias, 2013; Sarro, Wilson, & Sullivan, 2014; Weaver, 2007; Weaver et al., 2004). In human children, breastfeeding has been shown to play a prominent role in the promotion of healthy brain and

cognitive development. For example, there is a wealth of longitudinal research suggesting that longer duration of exclusive breastfeeding (EBF)[4] in infancy has beneficial effects on health in general but also on cognitive and intellectual development far into adult life (Daniels & Adair, 2005; Kramer et al., 2003; Oddy, 2002; Raju, 2011). Very recently, this has been qualified by the discovery that many neural outcomes, including total brain volume, cortical thickness, and white matter volume, are facilitated by the duration of EBF experience (Deoni et al., 2013; Isaacs et al., 2010; Kafouri et al., 2013).

While it is widely accepted that EBF is linked to improved cognitive development, most likely through its effects on neurodevelopment, very little is known regarding its potential impact on socio-emotional development (but see Hayatbakhsh, O'Callaghan, Bor, Williams, & Najman, 2012; Peus et al., 2012). This is quite surprising, considering that breastfeeding is much more than simply a meal at the breast- it is generally considered to be a dynamic biological and psychological process that is fundamentally social in nature (Raju, 2011; Uvnäs-Moberg, 1998). Moreover, breastfeeding is intricately linked to the hormone oxytocin, which is required to stimulate the let-down of milk (Dawood et al., 1981). Oxytocin is also present in small quantities in human milk, and released during pleasant touch and warmth (Uvnäs-Moberg, 1998). Although cerebrospinal oxytocin levels in human infants have not been measured, animal research suggests that oxytocin is released in response to suckling (Lupoli et al., 2001). Oxytocin acts as a neuropeptide in the brain, and has been implicated in a wide variety of social processes and behaviors, particularly those related to affiliation and bonding (Carter, Williams, Witt, & Insel, 1992; Feldman, 2012b; Young & Wang, 2004).There is accumulating evidence to suggest that oxytocin has specific effects on the perception of *positive* emotional expressions in adults (Gamer & Buchel, 2012; Guastella, Mitchell, & Mathews, 2008; Marsh et al., 2010). Nevertheless, the

[4] EBF refers to the amount of time an infant was fed with breast milk (from the breast and not from a bottle) as the *only* source of sustenance; e.g. EBF stops when the child is introduced to solid foods (Kramer et al., 2003).

exact mechanisms through which oxytocin impacts emotion perception are still being debated (Bartz, Zaki, Bolger, & Ochsner, 2011; Kemp & Guastella, 2011).

With respect to the link between exclusive breastfeeding and emotion perception, a recent study by Krol and colleagues (2014) showed that the duration of EBF had a similar effect on positive emotion recognition in mothers as the intranasal administration of oxytocin in other groups of adults (Domes, Steiner, et al., 2013; Marsh et al., 2010). Specifically, Krol and colleagues (2014) were able to demonstrate that longer EBF predicted better (faster) recognition of happy facial expressions. This suggests that EBF is linked to improved processing and recognition of affiliative cues such as positive facial expressions and that this processing advantage may be due to increased oxytocin levels.

4.1.3 The current study

From a developmental perspective, it is an open question whether exclusive breastfeeding has an effect on emotion perception in infants and if so whether this effect is similar to what has been shown in mothers (Krol et al., 2014). Therefore, the current study sought to examine the effect of EBF duration on the neural processing of emotional information in eight-month-old infants. We chose to investigate the Negative central (Nc) ERP component, which has been used to indicate attention allocation in infants. Our rationale for using ERPs was that (a) this method has been used repeatedly and reliably to elucidate emotion perception in nonverbal infants, and (b) ERPs and in particular the Nc have shown to be sensitive measures of individual differences in emotion perception in infancy (de Haan et al., 2004; Grossmann et al., 2011; Martinos et al., 2012). More specifically, the Nc is an electrophysiological correlate of infants' allocation of attention and general orienting to a visual stimulus occurring around 400-800 ms post stimulus onset, most prominently observed as a negativity at frontal and central electrodes (Courchesne, 1977, 1978; Courchesne et al., 1981; Nelson, 1994; Nelson & Dukette, 1998; Nelson & Monk, 2001). There is evidence showing that under certain conditions the Nc is comprised of two peaks

(Čeponienė et al., 2004; de Haan, 2007b; Karrer et al., 1998)- an early peak occurring around 300 to 800 ms, and a later peak occurring from around 800 ms. To date, only one study has examined the neural underpinnings of emotion discrimination from static body expressions in infants. Missana and colleagues (2014) found evidence for discrimination of emotion within the late Nc peak, but not within the early peak. We therefore chose to focus our analysis on the same time window (700-800 ms) as used in prior work. For this reason, we will refer to our specific time window as the "late Nc".

As in prior work, we presented infants with happy and fearful body expressions in an upright and inverted orientation (Missana et al., 2015; Missana et al., 2014). The inverted stimulus presentation was used as a control because body inversion has been shown to disrupt emotion recognition in adults (Atkinson et al., 2004) and emotion discrimination in infants (Missana et al., 2015; Missana et al., 2014; Zieber, Kangas, Hock, & Bhatt, 2014). On the basis of prior work with oxytocin (Domes, Steiner, et al., 2013; Marsh et al., 2010), we predicted that the duration of EBF is associated with differences in positive (happy) emotion processing. Specifically, similar to what has been shown for mothers (Krol et al., 2014), we hypothesized that infants who were exclusively breastfed for a longer duration would show an increased neural sensitivity to positive (happy) expressions. This neural sensitivity would be manifested by a larger, more negative late Nc. Importantly, we assessed a number of other variables that might be linked to breastfeeding and emotion perception, such as infant temperament, maternal dispositional interpersonal reactivity (empathic concern), parity, and maternal education. Focusing our investigation on differences in the duration of exclusive breastfeeding allowed us to examine variation within a group of breastfed infants, as opposed to comparing bottle-fed infants to breastfed infants.

4.2 Methods

4.2.1 Participants

The final sample included 28 infants (15 females) of European descent aged 243-261 days (M = 250.39, SD = 4.031) (about 8.23 months old), 13 of which whose data appear in an already published sample (Missana et al., 2014). An additional 10 infants were tested, but were excluded from the final sample due to a lack of questionnaire data (N = 4), fussiness (N = 2), artifacts (N = 2), and experimenter error (N = 2). The infants were born full-term (between 37 and 41 weeks) and had a normal birth weight (> 2500 g). Six infants were delivered via caesarean section and the rest underwent standard vaginal deliveries. All mothers that participated in our study had been on maternity leave up to the time of testing. All mothers provided informed consent prior to participation and were compensated with travel money and a toy for the infant. Procedures were approved by the ethics committee of the Leipzig University Medical School.

4.2.2 Stimuli

Stimulus material consisted of full-light static body expressions portraying six fearful and six happy expressions in both upright and inverted orientations. Still frames of a previous dataset of dynamic body expressions were selected at the peak of emotional expression (Atkinson et al., 2004; Missana et al., 2014) (Figure 4.1). From the original set of eight stimuli per condition, six were chosen for each emotion on the basis of recognition rate by a group of adult raters, indicating at least 40% average correct identification of the displayed emotion (chance level was 16.7%).

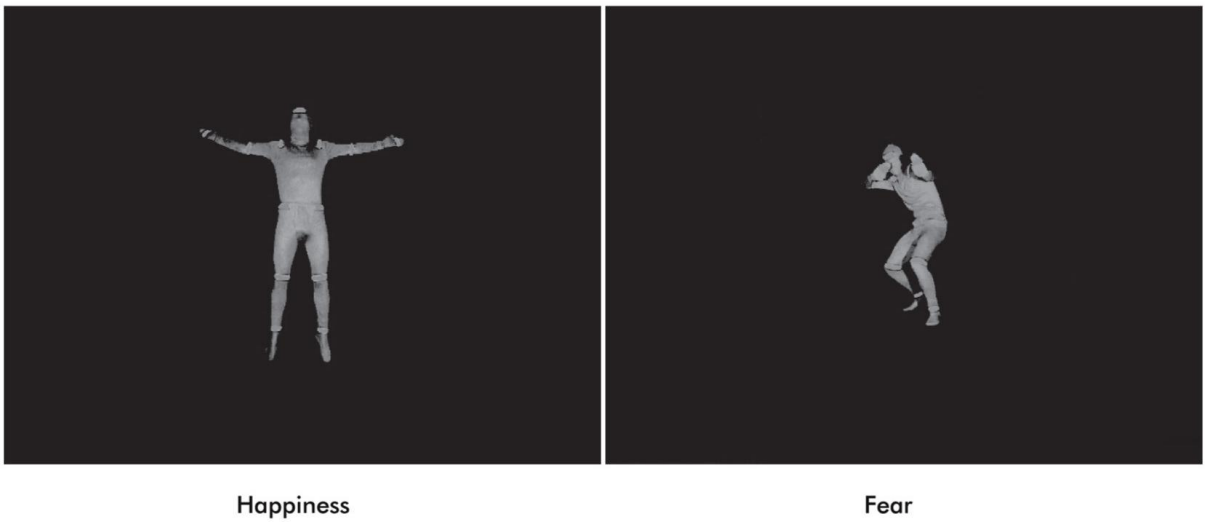

Figure 4.1 Examples of full-light static body expressions of happiness and fear.

4.2.3 Procedure

Infants were seated on their mothers' laps in a dimly lit, electrically shielded, and sound-attenuated room during stimulus presentation. Mothers were instructed to focus their attention on the child and to ignore the presented stimuli. Stimuli were displayed on a 70 Hz, 17-inch computer screen with a distance of about 70 cm. Images were presented in the center of the screen with a black background. Each image was preceded by an alerting sound in order to attract infants' attention. Each presentation trial began with a fixation cross (1000 ms) followed by a black screen (400 ms), followed by the stimulus for 2000 ms. An abstract screensaver was presented during the inter-stimulus interval in order to obtain the infants' attention for the subsequent trial. Sessions were video recorded online in order to ensure infants were looking at the screen. Trials were presented manually by button press. Stimuli were presented in a randomized order with the exception that no two stimuli of the same emotion/orientation combination were presented consecutively. The EEG session ended when the infant became fussy or inattentive. An average of 15.53 trials were presented per condition.

4.2.4 ERP analysis

EEG was recorded from 27 Ag/AgCl electrodes attached to an elastic cap designed for infants using the 10-20 system of electrode placement (EasyCap GmbH, Germany). Electrophysiological data was online referenced to the central CZ electrode and re-referenced offline to the average of the left and right mastoid electrodes. The horizontal electrooculogram (EOG) was taken from two electrodes (F9 and F10) located on the outer canthus of each eye. Vertical EOG was recorded from an electrode on the supraorbital ridge (Fp2) and an additional electrode placed on the infraorbital ridge of the right eye. EEG was amplified using a Porti-32/M-REFA amplifier (Twente Medical Systems International) and digitized at a rate of 500 Hz. Impedences were kept between 5 and 20 kΩ. Data processing for subsequent ERP analysis was performed with an in-house software package EEP, commercially available as EEProbeTM (Advanced Neuro Technology, the Netherlands). Raw EEG data was bandpass filtered between 0.3 and 20 Hz. Recordings were segmented into epochs which were time-locked to the stimulus onset, lasting from 200 ms before the onset until the offset of each stimulus (a 2200 ms duration). Epochs were baseline-corrected by subtracting the average voltage of the 200 ms baseline period prior to image onset. Epochs were rejected offline if the standard deviation within a gliding window of 200 ms exceeded 80 μV in any two of the bipolar EOG channels and 60 μV at EEG electrodes. Artifact-free epochs were averaged at each electrode for each condition (happy upright, happy inverted, fearful upright, and fearful inverted) in order to acquire ERPs. Each infant contributed an average of 5.5 artifact-free trials per condition (the average trial number did not differ between EBF groups: low EBF: 5.9, high EBF: 5.2). In a recent infant ERP methods review, Hoehl and Wahl (2012) demonstrate that 5 trials are sufficient to evoke clear Nc responses in infants using similar visual stimuli and working with infants of a very similar age as in our study.

A time window of 700-800 ms was chosen to investigate the late ERP negative central component (Nc) in the right hemisphere. The selection of the time window was based on prior

work (Missana et al., 2014) showing that Nc effects of emotional body expressions are seen later than in prior ERP work using emotional facial expressions. The selected time window still falls within the range of the commonly studied time range for investigating effects on the Nc (400-800 ms) (Courchesne et al., 1981). As stated in the introduction, some studies have found a two-peaked Nc (Čeponienė et al., 2004; Karrer et al., 1998). The ERP waveforms presented in Figure 4.2 indicate that the Nc is comprised of two peaks in the longer duration of EBF group. Mean amplitude effects were extracted from two ROIs: right fronto-central electrodes (F4, C4) and left fronto-central electrodes (F3, C3).

4.2.5 Breastfeeding questionnaire and analysis

A 10-item breastfeeding questionnaire was created in order to obtain demographic information as well as the following measures: the amount of time a mother *exclusively* breastfed her child (providing her child breast milk from the breast as the only source of sustenance), at what age the child was first introduced to other foods (if at all), and/or at what age the mother stopped breastfeeding the child. Whether the mother had breastfed in the last 24 hours was also recorded. The questionnaire provided a table in which mothers could describe their infants' feeding schedule (what time of day and what food intake) over the course of a normal day. Through this, frequency of breastfed meals per day could be calculated, as well as the percentage of daily meals that were breastfed. Additional information such as the age and parity of the mother, education, job, and immigration history was also collected. Durations provided in months (i.e. six months of EBF) were converted into days. If mothers were still exclusively breastfeeding, the infants' age on testing day was used in order to be as specific as possible. The duration of EBF was normally distributed in our sample and showed no significant skewness or kurtosis, M = 150.55 days, SD = 65.18. A mean split was obtained from EBF duration in order to create categorical groups of low and high EBF for further analysis and visualization (low EBF: M = 102.66 days, SD = 53.21; high EBF: M = 198.43 days, SD = 32.45).

4.2.6 Additional questionnaires

The revised Infant Behavior Questionnaire (IBQ-R) (Gartstein & Rothbart, 2003) was used to assess general infant temperament. This questionnaire is known internationally for its ability to gain insight into the unique temperaments of infants, including subscales assessing infant expression of smiling and laughter, approach tendency, perceptual sensitivity, cuddliness, fear, rate of recovery from distress, and many others. Additionally, the Interpersonal Reactivity Index (IRI) (Davis, 1983b) was given to mothers to assess dispositional empathy. This questionnaire includes four subscales, assessing the tendency one has to take the point of view of others (PT), the tendency to experience feelings of sympathy and compassion for those less fortunate (EC), the tendency to experience feelings of distress in response to discomfort in others (PD), and the tendency to transpose oneself into fictional situations such as novels or movies (FS).

Table 4.1 Breastfeeding descriptives.

	EBF low (*N* = 14)		EBF high (*N* = 14)			Total (*N* = 28)	
	Range	*M(SD)*	*Range*	*M(SD)*	*p*-value	*Range*	*M(SD)*
EBF duration (days)	12–152	102.66 (53.2)	167–253	198.43 (32.5)	<0.001	12–253	150.55 (65.2)
Breastfed meals/day (%)	0–50	7.20 (15.4)	0–87.50	35.31 (28.6)	0.003	0–87.50	21.25 (26.7)
Other children (#)	0–1	0.43 (0.5)	0–1	0.36 (0.5)	*ns*	0–1	0.39 (0.5)
C-sections (#)	*na*	0.21 (0.4)	*na*	0.23 (0.4)	*ns*	*na*	0.22 (0.4)
Education (years)	13–22	17.12 (3.2)	13–22	16.86 (2.4)	*ns*	13–22	16.98 (2.8)
Maternal age	26–35	30.93 (3.2)	26–37	31.51 (3.5)	*ns*	26–37	31.22 (3.3)

4.3 Results

The breastfeeding characteristics of the sample are presented in Table 4.1. Out of our 28 infants, 14 were still breastfed at least one meal a day. There were no EBF group differences in birth weight or gestation duration ($F(1,26) = .438$, $p = .514$, $\eta^2 = .017$; $F(1,26) = .122$, $p = .729$, $\eta^2 = .005$, respectively). Nor were there differences in maternal education or parity ($F(1,26) = .056$, $p = .816$, $\eta^2 = .002$; $F(1,26) = .140$, $p = .712$, $\eta^2 = .005$). EBF duration was not significantly

impacted by delivery method (caesarean section or vaginal delivery) ($F(1,25) = .177$, $p = .678$, $\eta^2 < .001$). Furthermore, there were no EBF group differences across any of the IBQ-R infant temperament subscales or maternal dispositional empathy as assessed through the IRI (all p-values > .05). Note that correlations with EBF as a continuous variable did not reveal any significant covariates (all p-values > .05). However, there is some evidence that maternal education might be a predictor of EBF duration (DiSantis, Hodges, & Fisher, 2013). We therefore included years of maternal education as well as current breastfeeding exposure (percent of breastfed meals/day) as covariates in all subsequent analyses.

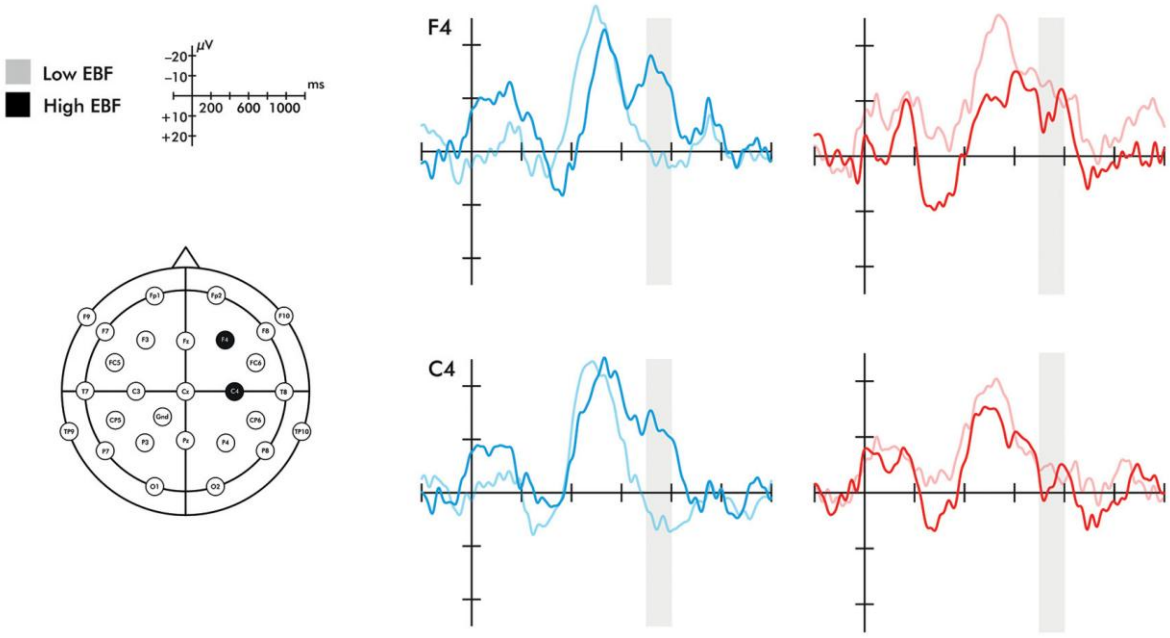

Figure 4.2 Event-related potentials for the F4 and C4 electrodes in response to happiness and fear. Region shaded in gray represents the time window of the Nc analyzed. Please note that the time windows of 0-200 ms and 400-600 ms did not differ significantly between groups for either electrode.

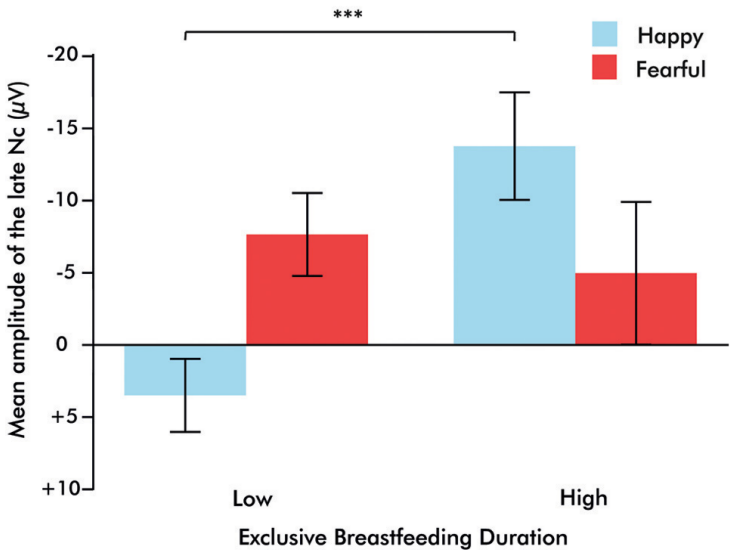

Figure 4.3 Bar graph illustrating the emotion x exclusive breastfeeding experience (EBF) interaction, in which infants with high EBF duration demonstrate a greater Nc to happiness than to fear, and infants with low EBF duration demonstrate a greater Nc to fear than to happiness. Bars represent standard error of the mean, ***p = 0.001.

A two (emotion: fearful, happy) x two (orientation: upright, inverted) x two (EBF experience: high, low) repeated measures ANOVA was conducted on the averaged right fronto-central (F4 and C4) late Nc. This analysis revealed a significant emotion x orientation x EBF experience interaction (F(1, 20) = 7.134, p =.015, η^2 =.263). To explore this three-way interaction further, 2 (emotion: fearful, happy) x 2 (EBF experience: high, low) repeated measures ANOVAs were conducted on the late Nc for upright or inverted expressions separately. This analysis yielded a highly significant emotion x EBF interaction in the upright condition (F(1,24) = 14.444, p = .001, η^2 = .376; Figures 4.2 and 4.3). Infants in the low EBF group showed greater (more negative) late Nc responses to fearful expressions than to happy expressions, suggesting a greater allocation of attention to fearful stimuli. In contrast, high EBF infants displayed greater late Nc responses to happy expressions and more positive late Nc responses to fearful expressions, suggesting greater attention towards happy stimuli. Further exploration of this interaction revealed that group differences in emotional processing were driven specifically by the late Nc to *happiness*, as group averages did not differ significantly for fear (happiness: F(1,26) = 14.667, p =.001, η^2 =.361, fear: F(1,26) = .220, p = .643, η^2 =.008 (difference for happiness survives

Bonferroni correction at $p < .025$)). Critically, there was no interaction between emotion and EBF experience in the inverted condition, $F(1,20) = 4.487$, $p = .237$, $\eta^2 = .069$, suggesting that the interaction effect is specific to emotional stimuli presented in an upright orientation. Note that, in line with prior work showing that the detection and discrimination of emotional body expressions is more prominent over the right hemisphere (Missana et al., 2015; Missana et al., 2014), no emotion x orientation x EBF duration interaction was observed for frontal and central electrodes (F3 and C3) over the left hemisphere ($F(1,20) = 3.457$, $p = .078$, $\eta^2 = .147$).

Table 4.2 Multiple regression indicating a positive prediction of EBF duration on the Nc in response to fearful expressions minus Nc in response to happy expressions.

	B	SE B	β	*t*	*p*-value
EBF duration (days)	−0.17	0.06	−0.56	−2.75	0.01
Breastfed meals/day (%)	0.22	0.16	0.29	1.37	0.19
Education (years)	0.44	1.35	0.06	0.32	0.75

Note: R^2: 0.244.

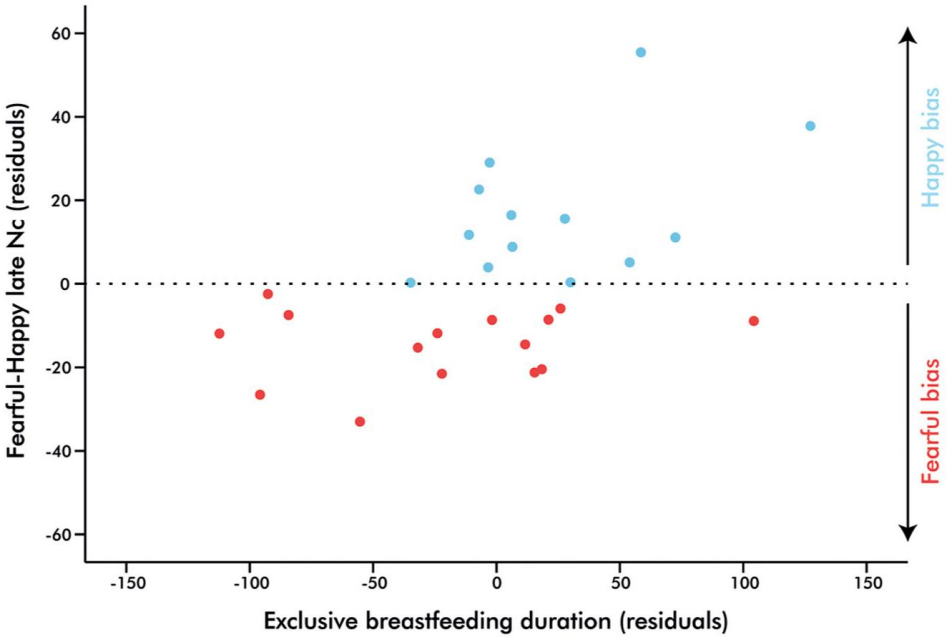

Figure 4.4 Partial regression plot illustrating the prediction of exclusive breastfeeding duration on infant brain response to fearful-happy expressions. Points above zero represent a bias towards happy stimuli, while points below zero represent a bias towards fear as indexed by the right fronto-central Nc (700-800 ms), $p = 0.01$.

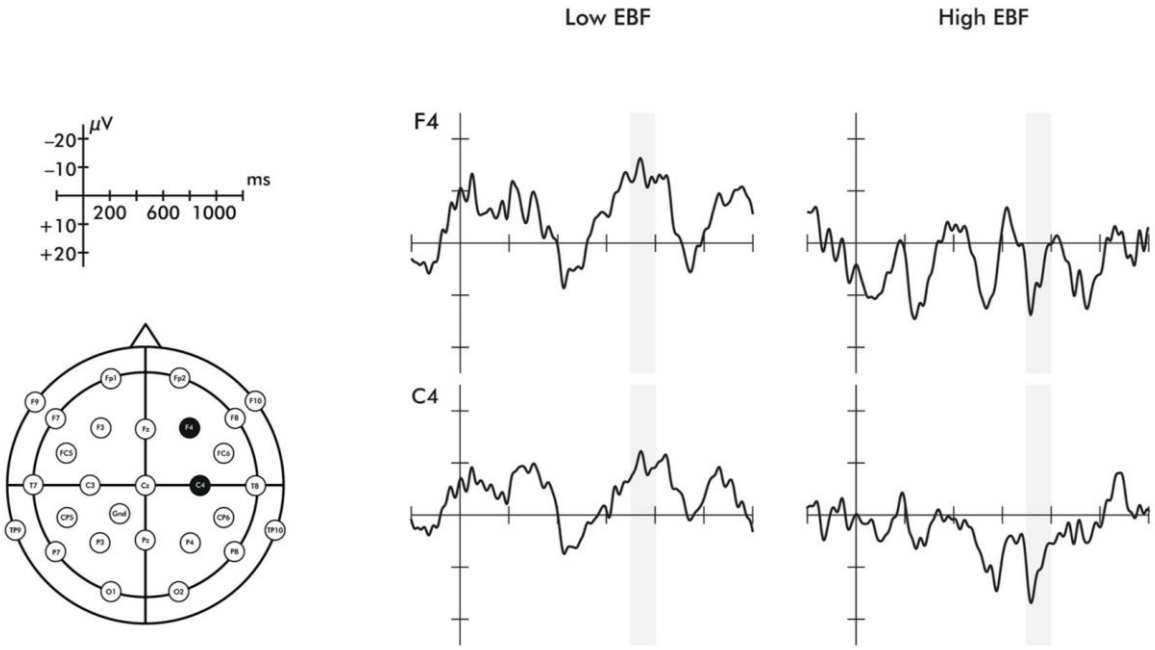

Figure 4.5 Event-related potentials for the F4 and C4 electrodes representing the response to fearful minus happy stimuli. Region shaded in gray represents the time window of the Nc analyzed. These are shown by exclusive breastfeeding duration (EBF) group for illustrative purposes, but note that the difference score was treated as a continuous variable in analyses.

Additionally, a difference score was computed in order to investigate a potential linear relationship between emotional biases of the late Nc amplitude with the duration of EBF. A forced-entry regression model was conducted in which EBF duration, maternal education, and current breastfeeding exposure predicted the fearful-happy Nc. EBF duration was the only significant predictor, suggesting that as days of EBF increase, the attentional bias towards fear shifts towards one for happiness (Table 4.2, Figure 4.4). Note that the impact of EBF on the fearful-happy Nc survives Bonferonni correction at $p < .0167$. The difference score waveforms are shown in Figure 4.5, separated by EBF group for visualization purposes.

4.4 Discussion

Our results revealed that exclusive breastfeeding duration was linked to differences in the neural processing of emotional body expressions in eight-month-old infants. Specifically, infants

with a high duration of exclusive breastfeeding showed a significantly greater late Negative central (Nc) component to happy body expressions than those with a short duration of exclusive breastfeeding, suggesting a greater allocation of attention to happiness with extended exclusive breastfeeding (EBF). This demonstrates that prolonged EBF in infants is associated with an increased sensitivity to positive emotional information in a similar fashion as shown in prior work with mothers (Krol et al., 2014). Such an increased sensitivity to positive emotional signals in both infants and mothers may be important in fostering positive social interactions and thereby might serve important affiliative and bonding functions in human development (Feldman, 2012a). Moreover, our analysis revealed that the bias toward positive or negative expressions shifted in a linear fashion as a function of EBF. Infants who were exclusively breastfed for a longer duration (above 152 days (approximately five months)) showed a positivity bias in their ERP responses (greater late Nc in response to happy than to fearful expressions), while infants who were exclusively breastfed for a shorter duration (below 152 days) showed a negativity bias in their ERP responses (greater late Nc in response to fearful than to happy expressions). This is the first evidence to suggest that emotion processing in infancy critically differs as a function of breastfeeding experience, supporting the notion that breastfeeding behavior is a complex biological and psychological process linked to early socio-emotional development.

Importantly, our results showed that the association between the duration of EBF and emotion processing in infancy exists independent of other variables that might have impacted the duration of EBF. In particular, we ruled out that EBF is linked to any of the following variables in our sample: several aspects of infant temperament (i.e. smiling and laughter, fear), maternal dispositional interpersonal reactivity (i.e. empathic concern), and parity. Current breastfeeding exposure (the percent of breastfed meals per day) and maternal education (in years) were factored into every analysis to further validate specific results of EBF. Furthermore, the association with EBF was not observed when the emotional stimuli were presented upside-down, which has been shown to disrupt emotion discrimination and recognition (Missana et al., 2015; Missana et al.,

2014), but was specific to emotional body expressions presented in the upright orientation. This strengthens our findings by showing that the association with EBF is specific to when emotional information can be detected in the stimulus.

An important point for discussion is related to the finding that the ERP differences between EBF groups were evident for a relatively late time window of the Nc. Specifically, infants in the longer duration of EBF group showed an additional negative deflection (peak) that followed the main Nc. Interestingly, there is prior work showing that under certain conditions the Nc comprises two peaks (Čeponienė et al., 2004; de Haan, 2007b; Karrer et al., 1998). Specifically, while the first peak has been linked to initial attentional orienting, the later second peak is viewed as reflecting sustained attention, possibly as a function of the salience of the stimulus. This difference in the timing of these processes might also map onto distinct cortical sources as an early aspect of the Nc is generated in anterior cingulate cortex and medial frontal gyrus and a later aspect of the Nc is localized to the frontal pole (Reynolds & Richards, 2005). With respect to the current findings, this may indicate that infants with high EBF experience recruit additional brain processes related to sustained attention when viewing happy body expressions, reflecting the salience of positive expressions for this group. Future work using neuroimaging techniques that allow for the precise mapping of cortical activation, such as functional near-infrared spectroscopy (fNIRS), is needed to further examine the possibility that infants recruit such specific brain processes linked to the frontal pole under these conditions (see Grossmann & Johnson, 2010; Grossmann et al., 2008 for infant fNIRS work imaging prefrontal cortex including frontal pole).

A further point for discussion relates to the role that oxytocin may play in the development of the emotional processing biases described in the current study. Our finding of an increased sensitivity to positive emotional expressions in infants with prolonged exclusive breastfeeding is not only in line with what has been found in mothers (Krol et al., 2014) but also fits well with what has been shown with intranasal administration of oxytocin (Domes, Steiner, et

al., 2013; Marsh et al., 2010). Furthermore, animal research has shown that breastfeeding increases central oxytocin levels in both mother and offspring (Dawood et al., 1981; Lupoli et al., 2001) and is thought to have similar effects in humans (Uvnäs-Moberg, 1998). It is also important to note that genetic differences in the oxytocin system have been found to give rise to morphometric alterations in healthy human adults particularly in limbic regions including the hypothalamus, amygdala, and anterior cingulate cortex (ACC) (Tost et al., 2010). As the infant Nc is believed to arise from frontal cortical regions, and in particular, the ACC (Reynolds & Richards, 2005), this could point to a method in which chronic modulation of the oxytocin system might impact structural differences in the brain which mediate attention and emotional processing. Given the converging picture from the current study and prior work, we would like to tentatively suggest that EBF affects central oxytocin levels in infants and thereby impacts emotion processing. In future work, it is crucial that this proposal be tested more directly by obtaining genetic and physiological information regarding the oxytocin system.

Regardless of the exact mechanism by which breastfeeding contributes to individual differences in emotion processing, there is also a need to discuss the current findings with respect to the general role that breastfeeding behavior may play in socio-emotional functioning. More specifically, the current findings have shown that infants' responsiveness to emotional information varies as a function of their breastfeeding experience and that biases towards positive and negative information are linked to the duration of EBF. Prior work demonstrates that, on average, infants begin to display a negativity bias around seven months of age, particularly seen as an increased attention and neural sensitivity to fearful facial expressions (Vaish et al., 2008). Our results suggest that this general developmental trajectory might be modulated by exclusive breastfeeding experience. Interestingly, the neural response to happy body expressions elicited in the high exclusive breastfeeding group appear similar to that of the average neural response to fearful body expressions found by Missana and colleagues (2014) in prior work as well as the infants in the low breastfeeding group. While this suggests that infants' allocation of attention to

emotional stimuli may differ depending on their breastfeeding experience, we would like to caution against the interpretation that infants in the high breastfeeding group somehow perceive happy expressions as fearful or threatening. This is because the neural index used in this study (late Nc) simply provides a correlate of attention allocation but does not tell us about the emotional valence of the perceived stimulus.

To understand why it is that some infants show what has been referred to as a negativity bias, while others show a positivity bias can help inform accounts of socio-emotional development by providing insights into the experiential dependence of emotional functioning (see Vaish et al., 2008). There are at least two possibilities to functionally interpret the current findings. First, the duration of EBF and the (assumed) exposure of the infant to increased levels of oxytocin may, during this sensitive phase of emotional development, have an early programming effect. Infants who are exclusively breastfed longer may become more sensitive to positive information, while infants who are exclusively breastfed for shorter durations may become more sensitive to negative information. This view is in agreement with work showing that maternal behavior can program stable changes in gene expression and neurobiological functioning that provide the basis for individual differences in socio-emotional behavior of the offspring (e.g., Kappeler & Meaney, 2010). Second, the cessation of EBF is often linked to the mother's effort to wean a child. Therefore, it is possible that the impact of EBF duration on emotion processing may reflect a consequence of late weaning. In this scenario, the later weaned infant may rely more on the mother, rendering negative or threatening information less important to attend to. Relatedly, earlier weaning may engender processes that sensitize the infant to negative information, as weaning is a first correlate of more independent functioning. This may thus be reflected in the way negative information is approached. Importantly, these two frameworks for explaining the current findings make rather different but testable developmental predictions. According to the programming account, infants that show a negativity or positivity bias at 8 months should still show the same kind of bias during later stages of development. In

contrast, according to the late weaning account, infants that show a positivity bias at 8 months would also start showing a negativity bias once they are weaned. It is thus of great importance to investigate the impact of EBF on socio-emotional functioning across development in longitudinal studies. Animal research will also help to explore how maternal nursing behavior impacts the neural and social development of their young. For example, recent work by Sarro and colleagues (2014) reports that nipple attachment and milk ejection impact the cortical synchrony of rat pups. These novel findings suggest possible mechanisms through which prolonged maternal care might induce robust individual differences in neural maturation.

While the current findings speak to the impact that maternal behavior has on infant social brain function, it is important to note that infants showed differential effects on processing emotions in response to (female) strangers. This suggests that the breastfeeding experience with the mother, while certainly having specific effects on the relationship between infant and mother, is also linked to more general processing differences that can be observed in response to people's body expressions that are unfamiliar to the infant. To find that specific social experiences, especially early in development, can have general effects on social responsiveness, is in line with a host of work examining individual differences in early social development (Curtis & Cicchetti, 2013; Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005; Pollak & Kistler, 2002; Pollak & Sinha, 2002).

Even though we took great care in ascertaining the specific influence of EBF on emotion processing, it should be acknowledged that other factors that were not examined in the current study may be linked to EBF and/or emotion perception and may thus influence the effects. For example, co-sleeping impacts how often the infant is able to breastfeed at night (Buswell & Spatz, 2007), and early sleep patterns in general may influence later social development (Dahl, 1996). We also did not investigate maternal mood or history of postpartum depression. Analyses from another cohort of mothers (Krol et al., 2014) found no correlation of either positive or negative mood on EBF duration. However, it could be possible that the maternal mood state or physical

contact impact infants' perception of emotional expressions. It is also very possible that infant temperament might not only impact a mother's decision to continue or cease breastfeeding, but also might impact the infant's perception of emotional expressions. However likely, we did not find any influence of infant temperament in the current sample. Another point is that parental gesture behavior might influence how familiar infants are with emotional body expressions. Future studies might benefit from recording natural interactions between the mother and infant, such that data regarding physical touch, gesturing, and affect can be taken into account. Moreover, due to the nature of our hypothesis (exploring variation within a group of breastfed infants), our study lacks a control group. One suggestion for future research is to include a group of exclusively formula-fed infants matched for the time they were introduced to solid foods. Such a control would help to parcel out specific influences of breastfeeding.

Finally, it must be stressed that although we discuss the role oxytocin may play in the processes studied here, we cannot confirm that EBF duration and oxytocin levels are correlated in our sample. Indeed, this has yet to be confirmed in any sample of human infants, and we must rely on animal research for the time being (Lupoli et al., 2001). It must also be acknowledged that the act of breastfeeding is a dynamic activity, which impacts several hormonal systems, physiological states, and brain processes. For example, breastfeeding reduces hypothalamic-pituitary-adrenal (HPA) axis activity in mothers (Heinrichs et al., 2002) as well as increases activation of several limbic regions in the maternal brain (Kim et al., 2011). Moreover, breastfeeding involves skin-to-skin contact and pleasant touch, which has been found to influence emotion regulation, attention, joint engagement, and pain analgesia (Feldman et al., 2014; Gray et al., 2000), as well as reducing heart rate responses in infants (Fairhurst et al., 2014). Until directly tested, increased oxytocin exposure must only be considered a potential pathway (among others) that can account for our current results. Future work will benefit from the addition of hormonal and genetic markers in both mothers and infants in order to achieve a

more detailed and possibly more mechanistic understanding of the effects of EBF on socio-emotional processing.

In conclusion, the current study provides first insights into the role that exclusive breastfeeding plays in contributing to individual differences during the neural processing of emotional expressions in infancy. Our results demonstrate that infants who had been exclusively breastfed longer showed an increased neural sensitivity to positive (happy) body expressions, while infants who had less EBF experience showed an increased neural sensitivity to negative (fearful) body expressions. The finding that such biases in emotional information processing occur in the context of the psychological and biological processes associated with breastfeeding during infancy is testament for a need to better understand the impact that maternal care in general and breastfeeding in particular has on socio-emotional functioning in infancy.

Acknowledgements

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Chapter 5.
Study 3: Genetic variation in CD38 and breastfeeding experience interact to impact infants' attention to social eye cues[5]

Kathleen M. Krol[a], Mikhail Monakhov[b], Poh San Lai[c], Richard P. Ebstein[b], & Tobias Grossmann[a,d]

[a]Early Social Development Group, Max Planck Institute for Human Cognitive and Brain Sciences, Leipzig, Germany; [b]Department of Psychology, National University of Singapore, Singapore; [c]Department of Paediatrics, Yong Loo Lin School of Medicine, National University of Singapore, Singapore; [d]Department of Psychology, University of Virginia, Charlottesville, USA

Abstract. Attending to emotional information conveyed by the eyes is an important social skill in humans. The current study examined this skill in early development by measuring attention to eyes while viewing emotional faces in seven-month-old infants. In particular, we investigated individual differences in infant attention to eyes in the context of genetic variation (*CD38* rs3796863 polymorphism) and experiential variation (exclusive breastfeeding duration) related to the oxytocin system. Our results revealed that, whereas infants at this age show a robust fear bias (increased attention to fearful eyes), their attention to angry and happy eyes varies as a function of exclusive breastfeeding experience and genetic variation in CD38. Specifically, extended exclusive breastfeeding duration selectively enhanced looking preference to happy eyes and

[5]Krol, K. M., Monakhov, M., Lai, P. S., Ebstein, R. P., & Grossmann, T. (2015). Genetic variation in CD38 and breastfeeding experience interact to impact infants' attention to social eye cues. *Proceedings of the National Academy of Sciences USA, 112*(35), E5434-E5442.

decreased looking to angry eyes. Importantly, however, this interaction was impacted by *CD38* variation, such that only the looking preferences of infants homozygous for the C allele of rs3796863 were affected by breastfeeding experience. This genotype has been associated with reduced release of oxytocin and higher rates of autism. In contrast, infants with the CA/AA genotype showed similar looking preferences regardless of breastfeeding exposure. Thus, differences in the sensitivity to emotional eyes may be linked to an interaction between the endogenous (*CD38*) and exogenous (breastfeeding) availability of oxytocin. These findings underline the importance of maternal care and the oxytocin system in contributing to the early development of responding to social eye cues.

Significance. Maternal care plays an important role in the development of the offspring's social behaviors through the programming of relevant neural and hormonal systems. However, it is unclear how specific maternal behaviors, such as breastfeeding and genetic variation related to the oxytocin system, contribute to emerging social behaviors in human infants. We therefore examined infants' attention to emotional eyes. Our results revealed that infants with the genotype previously associated with decreased availability of oxytocin and an increased rate of autism were most affected by extended durations of exclusive breastfeeding. Namely, these infants showed increased attention to happy eyes and decreased attention to angry eyes. This finding suggests that breastfeeding experience enhances prosocial tendencies in infants that are genetically at risk for autism.

5.1 Introduction

Sensitive responding to emotions in others is a vital social skill that helps us relate to others, predict their actions, and coordinate our own behavior during social interactions. Given its critical importance for effective social functioning, it is not surprising that the ability to detect and distinguish between emotional expressions emerges early in human development. Findings from behavioral and neuroscience studies indicate that infants' ability to discriminate between positive and negative emotional expressions emerges in the first year of life (see Grossmann, 2013; Vaish et al., 2008 for reviews). For example, by around seven months of age, infants distinguish between fearful and happy facial expressions and show increased allocation of attention to fear (Peltola et al., 2009). This enhanced attention to fear in others marks the emergence of a "fear bias" in infancy, which is thought to orient and alert the infant to potentially threatening situations (Leppänen & Nelson, 2012; Vaish et al., 2008). Prior work with adults demonstrates that emotion perception and, in particular, fear detection (eyes wide open) rely heavily on information from the eye region and occur even in the absence of conscious perception (Adolphs et al., 2005; Baron-Cohen, 1995; Lewis & Edmonds, 2003; Whalen et al., 2004). The importance of eye cues for emotion perception has recently been studied in infants. In an event-related brain potential (ERP) study, seven-month-old infants were found to discriminate between fear and happiness on the basis of eye cues alone (Jessen & Grossmann, 2014). Similar to adults, seven-month-old infants discriminated between emotional eyes without consciously perceiving them (see Kouider et al., 2013 for more information regarding conscious and unconscious processing in infants).

Studies on autism spectrum disorder (ASD), a neurodevelopmental disorder characterized by severe social impairments, provide more evidence to suggest that attending to eyes plays an important role in early social development. In particular, there is evidence to suggest abnormalities in looking to the eyes in toddlers, adolescents, and adults with ASD (Jones & Klin,

2013; Klin, Jones, Schultz, Volkmar, & Cohen, 2002; and Spezio, Adolphs, Hurley, & Piven, 2007 respectively). However, it should also be mentioned that some studies do not find impaired looking to the eyes among individuals with ASD, which may be explained by differences in the stimuli used, in the age ranges tested, and in the symptom severity considered across studies (see Guillon, Hadjikhani, Baduel, & Rogé, 2014 for a review). A recent study by Jones and Klin (2013) investigated attention to eyes from two to 24 months in infants at low or high risk for autism (high risk: infants with ASD siblings). Among infants later diagnosed with autism, attention to the eyes while viewing videos of naturalistic caregiver interactions was initially present but began to decline between two and six months of age. Furthermore, attention to eyes measured over the course of 24 months negatively correlated with symptom severity. Thus, children later diagnosed with autism initially attend to eyes as much as typically developing infants but later in infancy exhibit a steady decline in attending to the eyes whereas typically developing infants maintain a constant level of attention to the eyes (Jones & Klin, 2013). This finding points to an important period in infancy during which attention to eyes may develop along different trajectories. Furthermore, the results from Jones and Klin (2013) support the notion that the development of reduced attention to eyes and eye cues during infancy may be one of the earliest detectable warning signs of autism (see also Senju & Johnson, 2009).

In both autistic and healthy populations, oxytocin has been related to orientation to eyes and to the detection of emotional states from the eye region (Auyeung et al., 2015; Guastella, Mitchell, & Dadds, 2008). Oxytocin is a neurohormone synthesized in the paraventricular and supraoptic nuclei of the hypothalamus, released both peripherally into the bloodstream and centrally within the brain (Landgraf & Neumann, 2004). It is best known for its role in mammalian reproductive and parental behaviors (Lincoln & Paisley, 1982; Pedersen et al., 1982). However, it serves much broader functions and has been implicated in the modulation of a host of social behaviors, with a special emphasis on cooperative behaviors based on empathy and trust (Carter, 2014; Rilling & Young, 2014). With respect to the current context, a recent study by

Auyeung et al. (2015) reported a facilitation of eye contact with oxytocin administration in a real-time social interaction. This increase in eye contact was exhibited in both autistic and healthy adults. Oxytocin administration has also been shown to increase eye gaze to static faces in adults with autism (Andari et al., 2010) and has further been linked to improved emotion recognition from the eye region of static images in adolescents with autism (Guastella et al., 2010). Moreover, the administration of oxytocin to children (Gordon et al., 2013) and adults (Domes, Heinrichs, et al., 2013) diagnosed with autism has been shown to selectively enhance brain responses to social stimuli compared with physical control stimuli. In healthy adults, oxytocin administration increases the duration and frequency of gaze to the eye region while viewing neutral faces (Guastella, Mitchell, & Mathews, 2008) and increases orienting of attention to emotional gaze cues (Tollenaar, Chatzimanoli, van der Wee, & Putman, 2013). It also facilitates recognition of mental states of others when provided with cues from the eye region (Domes, Heinrichs, Michel, et al., 2007). Moreover, there is considerable evidence suggesting that oxytocin plays a specific role in facilitating the processing of positive emotions and events (Domes, Steiner, et al., 2013; Gamer & Buchel, 2012; Guastella, Mitchell, & Mathews, 2008; Marsh et al., 2010). This increase in the salience of positive emotions might also be linked to, or explained by, a reduction of the salience of negative social stimuli (Di Simplicio et al., 2009; Kemp & Guastella, 2011; Kirsch et al., 2005; Parr et al., 2013). Taken together, oxytocin seems to increase the salience of prosocial positive stimuli, which may be facilitated by increased attention to the eye region during social interactions.

Genetic variation that affects oxytocin neurotransmission has been linked to individual differences in social behavior (see Kumsta & Heinrichs, 2013; Meyer-Lindenberg et al., 2011 for reviews). Specifically, the ectoenzyme CD38 (Cluster of Differentiation 38) is considered to play a major role in regulating social behavior due to its effect on the release of oxytocin (Jin et al., 2007). Knockout mice that lack the *Cd38* gene show severe social deficits (i.e., amnesia of conspecifics) and have been discussed as a rodent model of autism (Higashida, Yokoyama,

Kikuchi, & Munesue, 2012; Higashida et al., 2011). Indeed, research points to a reduced expression of *CD38* in the lymphoblastoid cell lines of autistic patients (Lerer et al., 2010). With respect to naturally occurring variation at this gene, a common single-nucleotide polymorphism (SNP) has been described that occurs on an intron of this gene in humans, *CD38* rs3796863. Genetic variation at this locus has been associated with autism (Higashida et al., 2012; Higashida et al., 2011). Specifically, the C allele is reportedly overtransmitted in populations with autism and also associated with lower *CD38* expression (Lerer et al., 2010; Munesue et al., 2010). Furthermore, ASD in individuals with the CC genotype is characterized by more severe symptoms, such as restricted, repetitive, and stereotyped patterns of behavior, than ASD in individuals carrying the A allele (Munesue et al., 2010). In healthy populations, individuals who are homozygous for the "risk allele" (CC) have lower plasma levels of oxytocin than CA/AA carriers (Feldman, Gordon, Influs, Gutbir, & Ebstein, 2013; Feldman et al., 2012). When presented with social stimuli (both faces and scenes), adults with the CC genotype exhibit slower reaction times, increased fusiform gyrus activation (Sauer et al., 2013), and increased amygdala activation (Sauer et al., 2013) compared with adults with the CA/AA genotypes. At the behavioral level, parents homozygous for the C allele have been shown to touch their infants less during a free play session than A allele carriers (Feldman et al., 2012). In summary, this literature suggests that genetic variation in CD38 is associated with systematic neural and behavioral differences in social functioning.

Apart from genetic variation within the oxytocin system itself, maternal behaviors that influence the exogenous availability of oxytocin might also contribute to individual differences in emotion processing, namely breastfeeding. Plasma oxytocin rises in mothers in response to suckling (Dawood et al., 1981), and a similar rise in oxytocin occurs within the mothers' milk itself (Takeda et al., 1986). Although not directly studied in human infants, breastfeeding has been suggested to increase oxytocin levels in infants directly through breast milk, as well as indirectly through caring touch and warmth (Uvnäs-Moberg, 1998). Research with dairy calves

and rat pups provides evidence that plasma oxytocin levels rise as a direct result of suckling (Lupoli et al., 2001; Takeda et al., 1986). For example, Takeda et al. (1986) demonstrated that concentrations of radioactively marked oxytocin injected into rat dams were found in both the plasma and gastric contents of neonates after suckling. The duration in which human infants are fed with breast milk exclusively (exclusive breastfeeding; EBF) is recognized to facilitate cognitive (Daniels & Adair, 2005; Kramer et al., 2003; Mortensen et al., 2002; Oddy, 2002; Raju, 2011) and neural development (Deoni et al., 2013; Isaacs et al., 2010; Kafouri et al., 2013). However, only recently, EBF has been linked to differences in emotion processing in both mothers (Krol et al., 2014) and infants (Krol, Rajhans, et al., 2015). In both studies, an extended duration of EBF was associated with increased sensitivity to happiness in others. These breastfeeding studies are in line with oxytocin administration studies, in which oxytocin has been found to increase sensitivity to positive expressions in others (i.e., Marsh et al., 2010). It is thus likely that the influence of EBF on positive emotion perception is related to increased oxytocin levels in both mothers and infants.

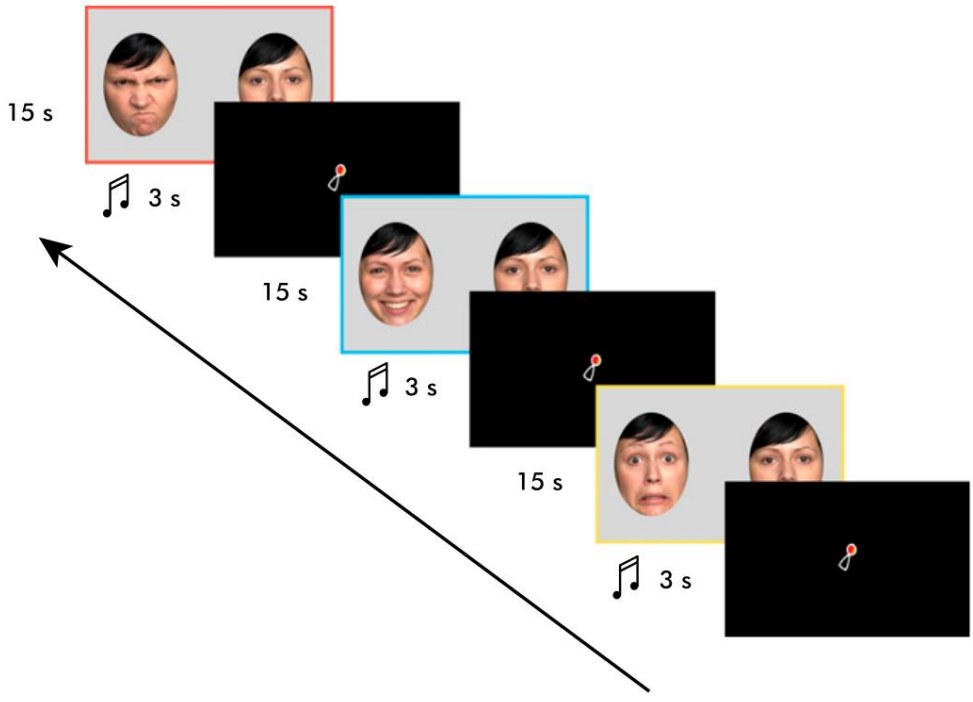

Figure 5.1 Presentation paradigm. Please note that the side in which the emotional face was presented was counterbalanced. Colored borders are shown to aid the reader in assessing the graphs below but were not presented to the infants. Stimulus duration is represented in seconds (s).

5.1.1 The current study

The goal of the current study was to systematically investigate infants' responses to emotional eyes and to examine how genetic variation in CD38 and variation in breastfeeding behavior impact individual differences in emotional eye processing. The specific aim of the current study was threefold: (*i*) to find out whether infants at seven months of age are able to differentially attend to emotional eye cues (and show a fear bias as seen for emotional faces), (*ii*) to examine whether exclusive breastfeeding duration, as a proxy for exogenously available oxytocin, impacts infants' attention toward the eye region of specific emotions, and (*iii*) to further investigate whether genetic differences related to central oxytocin release (endogenously available oxytocin) modulate attention to emotional eyes and whether and how this possible influence might interact with exclusive breastfeeding duration (see Figures 5.1 and 5.2 for presentation paradigm and stimuli examples). Based on prior work on emotional face perception in infancy, we hypothesized that seven-month-old infants are able to discriminate between emotions (Grossmann, 2013). More specifically, we predicted that infants would show increased attention to fear compared with other emotions (de Haan, Johnson, & Halit, 2003; Kotsoni et al., 2001; Nelson & de Haan, 1996; Peltola et al., 2009; Reynolds & Richards, 2005). We further hypothesized that duration of EBF is associated with differences in infants' attention to emotional information. As shown in prior work with infants and mothers (Krol et al., 2014; Krol, Rajhans, et al., 2015), we hypothesized that breastfeeding exposure would increase looking to happy eyes. Critically, we also hypothesized that the effect of EBF duration interacts with genetic variation within the oxytocin system. Specifically, based on prior work with rodents (Higashida et al., 2011) and both autistic and healthy humans (Feldman et al., 2012; Munesue et al., 2010), we predicted that the impact of breastfeeding might be greater for infants with the CC genotype (homozygous for the risk allele) of CD38 rs3796863. Because breastfeeding is thought to be an exogenous source of oxytocin (Higashida et al., 2011; Lupoli et al., 2001; Takeda et al., 1986), it might help up-regulate oxytocin levels (and function) in infants with the "risk" CC genotype

presumably characterized by low oxytocin levels. It is also important to mention that the choice and the extent of breastfeeding may be impacted by potentially confounding maternal factors such as maternal age and education (Bertini et al., 2003; Scott & Binns, 1999). The current study therefore obtained information regarding these attributes and other variables through questionnaires.

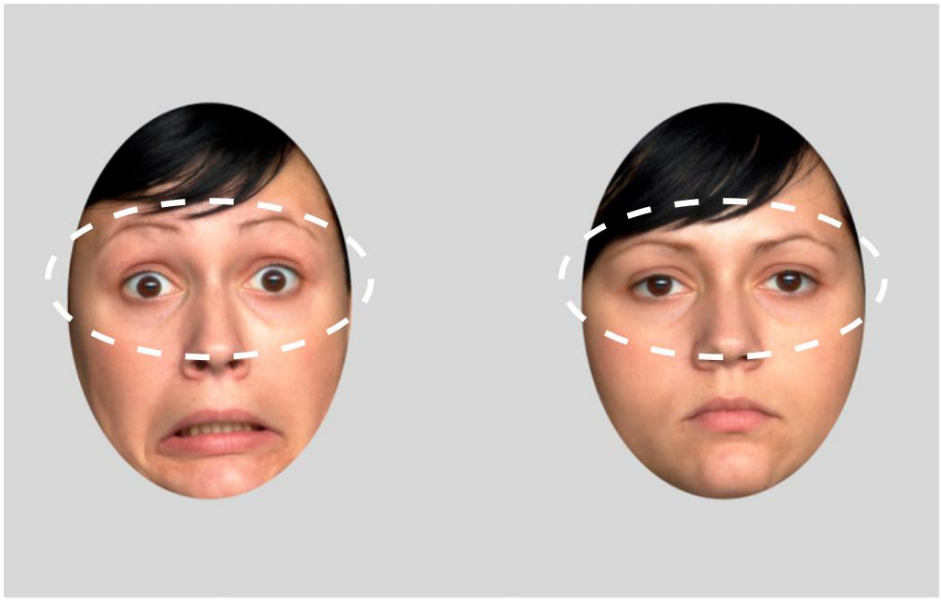

Figure 5.2 Regions of interest (ROIs). Dashed lines represent the ROIs analyzed for each emotion and actress.

5.2 Results

CD38 rs3796863 genotype frequencies in the infants are displayed in Table 5.1 and are similar to those observed in other studies (Sauer et al., 2013; Sauer et al., 2012). No Mendelian inconsistencies were detected in inheritance of alleles from mothers to offspring (see Table 5.4 for maternal genotype frequencies). Hardy–Weinberg equilibrium was tested separately in mothers and in offspring. No deviations from Hardy–Weinberg equilibrium were detected (all *p*-values > 0.05). Sex distribution and age differed significantly neither between genotype groups (CC versus CA/AA) nor between EBF groups (low versus high) (all *p*-values > 0.05). The

majority of studies relating the polymorphism rs3796863 (*CD38*) with aspects of social behavior and plasma oxytocin find differences between homozygous risk allele carriers (CC) and A carriers (CA/AA) (Algoe & Way, 2014; Feldman et al., 2013; Feldman et al., 2012; Sauer et al., 2013; Sauer et al., 2012). In keeping with prior studies, we conducted our analyses by grouping genotypes in the same manner (CC versus CA/AA).

Table 5.1 Infant *CD38* rs3796863 genotype frequencies.

Genotype	Frequency	%
CC	44 (18 F, 26 M)	44.9
CA	49 (27 F, 22 M)	50
AA	5 (4 F, 1 M)	5.1

F, females; M, males.

As our main analysis, we conducted a three (emotion: anger, happiness, fear) by two (EBF: low, high) by two (CD38: CC, CA/AA) repeated-measures analysis of covariance (ANCOVA) with looking preference to the eye region as the dependent variable. Covariates included in our analysis were maternal age, maternal education, and the percentage of currently breastfed meals (Table 5.5). Maternal age and education were included because prior work indicates that these factors are associated with breastfeeding behavior and should therefore be controlled as covariates (Daniels & Adair, 2005; Deoni et al., 2013; Isaacs et al., 2010; Kafouri et al., 2013; Kramer et al., 2003; Mortensen et al., 2002; Oddy, 2002). Based on prior work with infants who are still breastfed (Krol et al., 2014; Krol, Rajhans, et al., 2015), we also included currently breastfed meals as a covariate because it allowed us to ascertain that breastfeeding effects can be attributed to exclusive breastfeeding duration (EBF): that is, the prolonged breastfeeding experience, rather than current breastfeeding behavior as reflected in currently breastfed meals. The main and interaction effects from this omnibus ANCOVA analysis and the subsequent follow-up tests are reported in the following paragraphs.

5.2.1 Emotion

Our analysis revealed a main effect of emotion ($F(2,178) = 10.530$, $p < 0.001$, $\eta^2 = 0.106$). Post hoc pairwise comparisons revealed that infants showed an increased looking preference to fearful eyes ($M = 0.59$, $SEM = 0.020$) compared with happy eyes ($M = 0.49$, $SEM = 0.021$) and compared with angry eyes ($M = 0.47$, $SEM = 0.020$), Bonferroni-adjusted $p < 0.001$ and $p = 0.001$, respectively. Looking to happy eyes and angry eyes did not differ significantly (Bonferroni-adjusted $p = 1.00$).

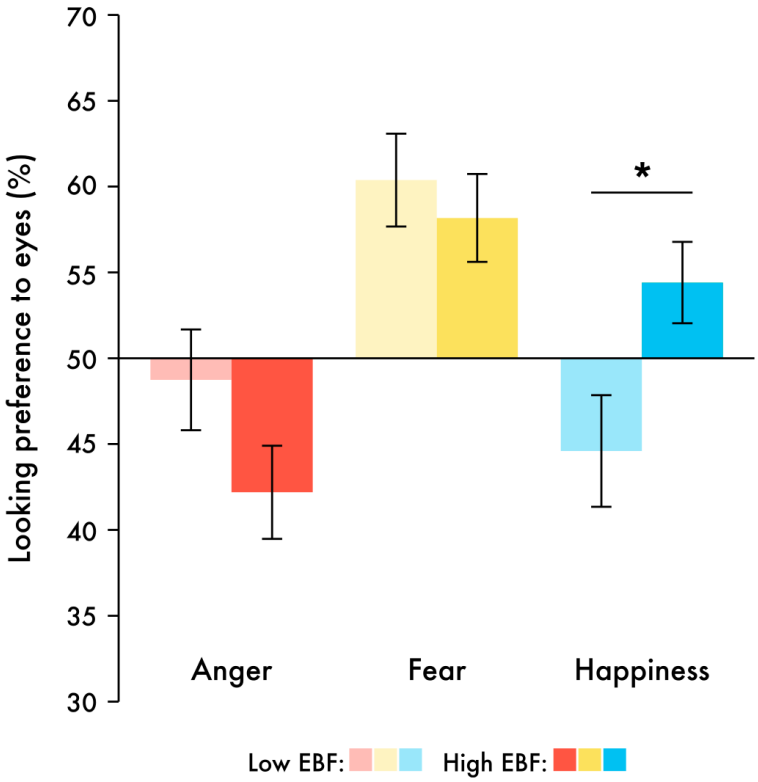

Figure 5.3 Bar graph illustrating the interaction between EBF and emotion. Infants with a high EBF duration displayed significantly longer looking preferences to happy eyes than infants with low EBF. *$p < 0.05$; $N = 98$; data are presented in raw form and bars represent ± *SEM*.

5.2.2 Emotion and breastfeeding

Our analysis further revealed a significant interaction between the factors emotion and EBF ($F(2,178) = 3.6$, $p = 0.029$, $\eta^2 = 0.039$). One-way ANOVAs conducted for the three emotions separately revealed that this interaction was driven by happiness. Specifically, infants in the high exclusive breastfeeding group showed higher looking preferences to happy eyes than infants in the low exclusive breastfeeding group ($F(1,96) = 5.591$, $p = 0.02$). Anger and fear looking preferences did not differ by breastfeeding experience ($F(1,96) = 2.613$, $p = 0.109$ and $F(1,96) = 0.343$, $p = 0.56$, respectively) (Figure 5.3).

Table 5.2 Regression analysis indicating a positive prediction of EBF duration on looking preference to happy eyes minus looking preference to angry eyes.

Model	*B*	SEM	β	*t* value	*P* value
(Constant)	0.025	0.031		0.826	0.411
EBF	0.089	0.032	0.282	2.821	0.006
Maternal education	−0.004	0.033	−0.013	−0.120	0.905
Maternal age	0.050	0.033	0.159	1.524	0.131

Model summary: $F(3,92) = 3.736$, $P = 0.014$, $R^2 = 0.109$.

As seen in Figure 5.3, it seems that EBF might impact attention to angry eyes in the opposite direction of that to happy eyes, such that infants in the high EBF group have a higher looking preference to happiness and a lower looking preference to anger than infants in the low EBF group. Therefore, we explored a potential linear relationship between EBF duration and the bias toward either happy eyes or angry eyes. A difference score was created ((looking preference to happy eyes) − (looking preference to angry eyes)) on which we could conduct a forced-entry regression. With EBF, maternal education, and maternal age as predictors, only EBF significantly predicted this difference score (Table 5.2). As the duration of EBF linearly increases, a looking preference toward angry eyes shifts toward one for happy eyes (Figure 5.5). This pattern might

also reflect an avoidance of angry eyes with increased EBF duration. For results of one-sample t-tests comparing each preference score to chance (50%), see Figure 5.6.

5.2.3 Emotion, breastfeeding, and CD38

Critically, our analysis also revealed a three-way interaction between the factors emotion, EBF, and CD38 genotype ($F(2,178) = 3.287$, $p = 0.04$, $\eta^2 = 0.036$). To explore this interaction further, three (emotion: anger, happiness, fear) by two (EBF: high, low) repeated-measures ANCOVAs were conducted separately for each of the two CD38 genotype groups. We found a main effect of emotion for both genotype groups (CC, $F(2,74) = 3.514$, $p = 0.035$, $\eta^2 = 0.087$; CA/AA, $F(2,98) = 6.378$, $p = 0.002$, $\eta^2 = 0.115$). However, the interaction between the factors EBF and emotion was only significant within CC carriers ($F(2,74) = 5.151$, $p = 0.008$, $\eta^2 = 0.112$) (Figure 5.4). Within the CC genotype, infants in the high exclusive breastfeeding group showed a greater preference for happy eyes (increased looking) and a greater avoidance of angry eyes (reduced looking) than infants in the low exclusive breastfeeding group (happiness, $F(1,42) = 7.886$, $p = 0.008$; anger, $F(1,42) = 4.613$, $p = 0.038$). In the CA/AA genotype group, no such effects were observed. The looking preference averages for each breastfeeding group and genotype are displayed in Table 5.3.

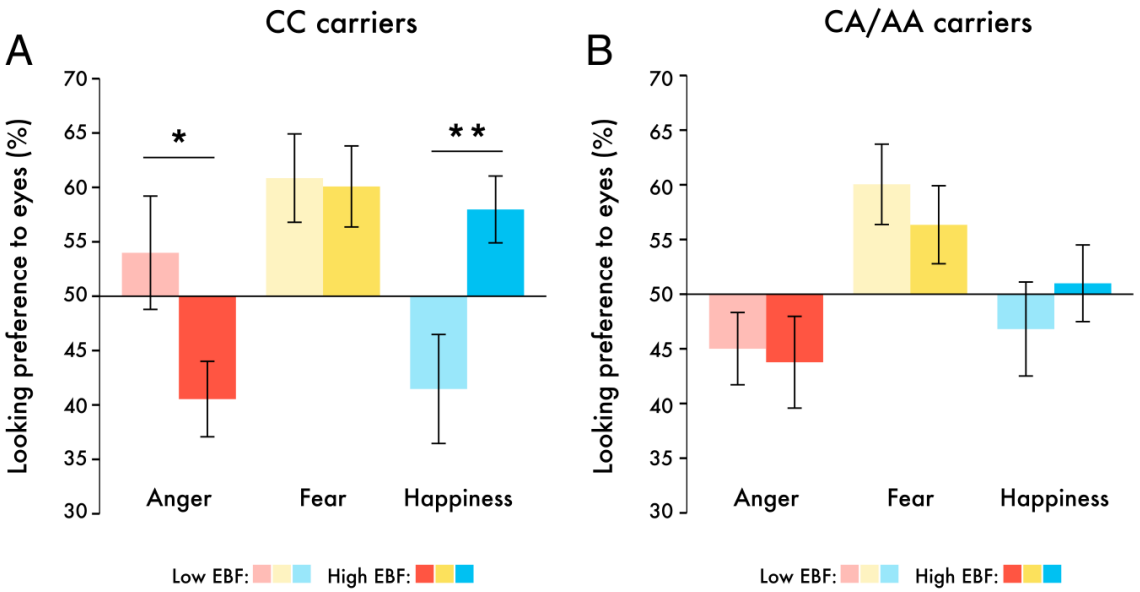

Figure 5.4 Infant looking preferences were modulated by an interaction between EBF and CD38 genotype. Bar graphs represent emotion and EBF interactions for (A) CC carriers and (B) CA/AA carriers separately. *$p < 0.05$, **$p < 0.01$; $N = 98$; data are presented in raw form and bars represent ± *SEM*.

Table 5.3 Looking preferences (%) for each emotion, split by genotype and breastfeeding groups.

	CC		CA/AA	
Emotion	EBF low	EBF high	EBF low	EBF high
Angry eyes	53.30 (4.21)	44.12 (4.40)	41.61 (3.58)	47.02 (4.08)
Fearful eyes	60.46 (4.24)	60.12 (4.42)	59.30 (3.60)	57.21 (4.10)
Happy eyes	38.79 (4.45)	60.40 (4.64)	45.69 (3.78)	51.51 (4.30)

Values are presented as mean (SEM).

5.2.4 Additional effects

Our analysis also revealed main effects of the covariates maternal education and currently breastfed meals ($F(1,89) = 6.494$, $p = 0.013$, $\eta^2 = 0.068$, and $F(1,89) = 4.502$, $p = 0.037$, $\eta^2 = 0.048$, respectively). Note that, despite the observed main effects of these covariates, removing them as covariates from the statistical model leaves the main findings reported from the omnibus repeated measures ANOVA above unchanged. To further explore the main effects of the two covariates maternal education and currently breastfed meals, we computed correlations with

looking preferences to emotional eyes for both maternal education and currently breastfed meals. The correlation analysis showed that only looking preference to angry eyes, but not happy or fearful eyes, correlated negatively with percentage of breastfed meals ($r(97) = -0.289$, $p = 0.004$) and with maternal education ($r(97) = -0.241$, $p = 0.017$). Specifically, the greater the maternal education and percentage of breastfed meals, the greater was infants' preference for neutral eyes compared with angry eyes, which might reflect an avoidance of angry eyes. With respect to percentage of breastfed meals, this result is similar to a study with mothers in which the percentage of breastfed meals slowed down reaction times when recognizing angry faces (Krol et al., 2014). Maternal education and percentage of breastfed meals showed a positive relation but were not significantly correlated with each other ($r(97) = 0.162$, $p = 0.113$). There were no interactions between covariates, and the covariates did not differ across genotype groups. Relevant data files have been deposited in the Center for Open Science digital repository (https://osf.io/6pnaj).

5.3 Discussion

In the current study, we examined infants' responses to emotional eyes in the context of common genetic variation within the *CD38* gene and variation in exclusive breastfeeding experience. Our analysis of infants' looking patterns yielded three main findings. First, as a group, seven-month-old infants showed a fear bias: increased looking toward fearful eyes compared with happy and angry eyes. Second, infants' increased breastfeeding experience was associated with enhanced looking to happy eyes and aversion of angry eyes. Third, these effects of breastfeeding on emotional eye processing were observed only in infants with the CC genotype of the *CD38* gene (rs3796863). Our findings suggest that differences in the sensitivity to emotional information conveyed through the eyes are linked to the oxytocin system. Specifically, the pattern of results points to an interaction between factors impacting the endogenous (*CD38*) and

exogenous (breastfeeding) availability of oxytocin. This finding underlines the importance of the oxytocin system in contributing to sensitivity to emotional cues from the eyes. We will now discuss the three main findings in turn.

5.3.1 Emotion

Our results show that, at the age of seven months, infants' looking to fearful eyes is significantly increased compared with happy and angry eyes. This finding demonstrates that, as a group, infants show what has been termed a fear bias (Leppänen & Nelson, 2012). This increased attention to fear in others is in line with prior behavioral and neuroscience work with infants of that age (de Haan et al., 2003; Kotsoni et al., 2001; Nelson & de Haan, 1996; Peltola, Hietanen, Forssman, & Leppänen, 2013; Peltola et al., 2009; Reynolds & Richards, 2005). Enhanced attention to fear has been argued to serve important adaptive functions because it is thought to alert the individual to potential dangers and threats in the environment (Peltola et al., 2013). Interestingly, although fearful and angry faces both represent negative expressions that might be associated with threat, our results show that infants dedicate more attention to fearful eyes than angry eyes. This outcome indicates that, rather than indiscriminately heightening attention to negative cues, infants selectively increase looking at the eyes of someone experiencing fear. This finding is in agreement with work in adults showing that fearful expressions elicit approach behavior and are linked to helping whereas angry expressions elicit avoidance behavior (Marsh, Ambady, & Kleck, 2005; Marsh, Kozak, & Ambady, 2007; Marsh et al., 2012). Therefore, selective enhanced attention to fearful eyes as seen in our infant data may reflect infants' approach and empathic concern for others in distress (experiencing fear) (Davidov, Zahn-Waxler, Roth-Hanania, & Knafo, 2013). Alternatively, infants may also look longer at fearful eyes because they expect gaze cues that help them identify the source of threat (Hoehl, Wiese, & Striano, 2008) or because they exhibit a heightened perceptual sensitivity to the large eye whites characteristic of fearful eyes (Jessen & Grossmann, 2014). Regardless of the exact nature of this

effect, the current findings provide evidence for the notion that infants of this age show a robust fear bias.

5.3.2 Emotion and breastfeeding

Our analysis further revealed an interaction between the factors emotion and exclusive breastfeeding duration (EBF). Importantly, however, the two-way interaction between emotion and EBF was further qualified by a three-way interaction including *CD38* variation and can therefore be fully interpreted only when taking into account infant *CD38* genotype. Before a detailed discussion of the role of *CD38* genotype, we nonetheless briefly summarize and discuss our findings with respect to variation in EBF because they replicate and critically extend prior work on that topic with infants and mothers. More specifically, our results show that infants who were exclusively breastfed for a longer duration exhibit increased looking to happy eyes than those infants exclusively breastfed for a shorter duration. This finding confirms our prediction based on previous work in which longer exclusive breastfeeding durations were associated with greater attention allocation in infants to happy body expressions as indexed by ERP measures (Krol, Rajhans, et al., 2015). This increased sensitivity to happiness in others as a function of exclusive breastfeeding duration is also seen in mothers (Krol et al., 2014), suggesting that infants' and mothers' responses to happiness are similarly affected by breastfeeding experience. Moreover, our regression analyses showed an association between currently breastfed meals and infants' responses to angry eyes. Specifically, as the percentage of breastfed meals increased, the looking toward angry eyes decreased. This effect of breastfeeding on looking away from angry eyes, although not statistically significant, seems to be in a similar direction also when considering duration of EBF (Figure 5.3). This aversion of angry eyes is similar to what has been found in mothers (Krol et al., 2014), in which the percentage of breastfed meals slowed down reaction time when recognizing angry faces. Such an increased sensitivity to positive emotional expressions in both mothers and infants might be important in fostering positive social

interactions and thereby might facilitate affiliation and bonding in human development (Feldman, 2012a). It is also in general agreement with accounts that assign an important role to maternal behaviors in the early development of social functioning (Hrdy, 2009; Kappeler & Meaney, 2010). It is also important to emphasize that the effects of the breastfeeding variables obtained in the current infant study resemble the effects of oxytocin administration in prior work on emotional face perception with adults (Domes, Steiner, et al., 2013; Marsh et al., 2010). Taken together, our findings indicate that increased breastfeeding experience, presumably through its effects on the oxytocin system, is associated with enhanced sensitivity to happy eyes and a decreased sensitivity to angry eyes. This interpretation is in line with accounts that view oxytocin's role in social functioning as facilitating approach while simultaneously reducing withdrawal tendencies (Kemp & Guastella, 2011).

5.3.3 Emotion, breastfeeding, and CD38

Whether and how the effects of breastfeeding are moderated by genetic variation related to the central release of oxytocin (*CD38*) will be discussed in this section. Addressing this question can shed light on how endogenous and exogenous variables affecting the oxytocin system interact in contributing to infants' sensitivity to emotional eyes. Our results show that the association between EBF duration and specific differences in emotional eye processing is present only in individuals homozygous for the C allele of *CD38* rs3796863. Specifically, among infants with the CC genotype (lower endogenous oxytocin), those with longer durations of EBF (higher exogenous oxytocin) experience exhibited enhanced looking to happy eyes and decreased looking (even looking away, avoidance) of angry eyes, compared with infants of the same genotype but with shorter durations of EBF. This finding was in line with our prediction based on prior work with rodents (Higashida et al., 2011) and both autistic and healthy humans (Algoe & Way, 2014; Feldman et al., 2013; Feldman et al., 2012; Munesue et al., 2010), implicating the CC genotype in impaired social functioning associated with reduced oxytocin levels. Critically, our results suggest

that breastfeeding, probably through its role as an exogenous source of oxytocin (Lupoli et al., 2001; Takeda et al., 1986), may help regulate oxytocin levels (and function) specifically in infants with the CC genotype who may have a greater risk for social dysfunction. It seems that low endogenous oxytocin, albeit genetically determined, can be corrected for in a sense by an environmental manipulation, namely, extended breastfeeding. This finding may have important implications regarding genetic hard wiring, and we suggest the notion that other instances of perceived genetic determinism can be minimized or neutralized by appropriate behavioral interventions, which in our study are represented by breastfeeding. This paper adds to the growing consensus that, although genes are important in human behavior, they are not deterministic.

More generally, the obtained results indicate that infants in the CC genotype group are more susceptible to the effects of their maternal environment (breastfeeding experience). To find such a pattern of differential susceptibility to breastfeeding experience on the basis of the genotype concurs with accounts stipulating that putative "risk genes" might in some instances be more appropriately conceptualized as "plasticity genes" (Belsky et al., 2009). Rather than reflecting a predisposed risk factor, some genes may essentially make the individual infant more susceptible to context, whereby the direction of the effect depends on the context. Namely, in the current study, in the CC genotype group, shorter durations of breastfeeding are associated with reduced attention to happy eyes and increased attention to angry eyes whereas longer durations of breastfeeding had opposite effects (increased attention to happy eyes and decreased attention to angry eyes). According to this view, the current results point to a potential plasticity gene (CC genotype) involved in regulating oxytocin effects on social functioning during early development related to responding to emotional eyes. A critical question that arises from this investigation is whether infancy represents a sensitive period in human development during which such plasticity is observed. It should also be considered that this period of plasticity may well extend into adolescence (Galván, 2014). Relatedly, it will be important to find out whether

the biases seen in seven-month-old infants' attention to emotional eyes based on the interaction between breastfeeding and *CD38* genotype endure beyond infancy and, if so, how they impact social behavior in children more globally. Ultimately, longitudinal work is needed to elucidate these critical questions.

5.3.4 Limitations and future directions

Apart from the need for longitudinal data to examine potential long-term effects, in future work, it will also be critical to investigate the neural processes that are involved in the interaction between breastfeeding and *CD38* genotype when accounting for individual differences in the attention to social eye cues. Prior work has shown that oxytocin exerts neurophysiological effects in the brain by directly targeting fast-spiking interneurons. Specifically, there is work demonstrating that the administration of oxytocin in rats increases throughput of output spikes, sharpens spike timing, and suppresses background firing, which improves circuit-level signal-to-noise ratios and can thereby increase the salience of certain stimuli (Owen et al., 2013). This signal-to-noise enhancement can be impacted by certain developmental events and experiences. For example, when female rats become dams, responses to pup distress calls become enhanced by balancing cortical inhibition, and the same effect can be mimicked in virgin female rats by administering oxytocin (Marlin, Mitre, D'amour, Chao, & Froemke, 2015). This literature supports the notion that oxytocin plays a role in enhancing social responsiveness. Moreover, as previously mentioned, the administration of oxytocin to children (Gordon et al., 2013) and adults (Domes, Heinrichs, et al., 2013) diagnosed with autism has been shown to selectively enhance brain responses to social stimuli compared with physical control stimuli. Interestingly, it has also been observed that, for some individuals with ASD, oxytocin administration is more effective in enhancing brain responses to social cues than in others (Gordon et al., 2013). Our infant data suggest that there are genetic factors, namely CD38 genotype, that may play a role in accounting for how effective exogenously stimulated oxytocin

levels enhance responding to social cues. It may thus be important to examine whether CD38 variation can also account for the effectiveness of oxytocin administration in ASD.

As a further consideration and limitation, it must be stressed that breastfeeding is a dynamic and complex process that involves and impacts several hormonal, physiological, and psychological systems (Raju, 2011). It is therefore difficult to unpack and to determine the exact mechanisms by which breastfeeding exerts the effects seen in the current study. For example, breastfeeding involves other behaviors such as physical contact associated with warmth and pleasant touch (Uvnäs-Moberg, 1998), both of which have been shown to, on the one hand, reduce heart rate in infants (Fairhurst et al., 2014) and, on the other hand, enhance emotion regulation, social engagement, and pain analgesia (Feldman et al., 2014; Gray et al., 2000). Furthermore, factors related to attachment quality, especially maternal sensitivity, have been shown to impact breastfeeding behavior, with increased maternal sensitivity related to prolonged breastfeeding rates (Britton, Britton, & Gronwaldt, 2006). These physiological, behavioral, and psychological variations are all important factors to take into account in future studies on the effects of breastfeeding on social functioning in infancy.

5.3.5 Conclusion

All in all, the current study demonstrates that differential attention to emotional eye cues, as a vital social skill, develops during infancy. Importantly, differences in this sensitivity to emotional eye cues emerge in the context of an interaction of factors associated with genetic variation in *CD38* and breastfeeding experience. This finding provides evidence that, early in human ontogeny, endogenous and exogenous factors involved in regulating oxytocin levels contribute to individual differences in emotional attention. This research points to early emerging biases in emotion processing that may plastically shape pathways of socio-emotional development.

5.4 Materials and Methods

5.4.1 Participants

Ninety-eight seven-month-old infants (49 females, 49 males) participated with their mothers in this study. Infants' age ranged from 204 to 232 days (M = 214 d, SEM = 0.56), and mothers' age ranged from 22 to 45 years (M = 31.49, SEM = 0.45). All infants were typically developing, had a normal birth weight (>2,500 grams), were born full-term (37–41 weeks), and were of European descent. There was no known history of autism spectrum disorder either in any of the participating mothers or in any of the older siblings. 78 infants had standard vaginal deliveries, and 20 were delivered via caesarean section. All but one infant had mothers that were still on maternity leave up to the time of testing. Informed consent was provided by the infants' parents before participation in the study. All procedures were approved by the Leipzig University Medical School Ethics Committee and were performed in accordance with the Declaration of Helsinki. Parents were reimbursed for travel, and infants received a toy and a t-shirt.

5.4.2 Breastfeeding

A questionnaire developed in house was completed by mothers to obtain detailed information on breastfeeding experience (Krol et al., 2014; Krol, Rajhans, et al., 2015). Mothers filled out a table that detailed a feeding plan for a typical day. The percentage of breastfed meals was calculated by dividing the number of breastfed meals by the number of total meals. Mothers also indicated whether they were still exclusively breastfeeding and, if not, at what age (infant) they ceased. Exclusive breastfeeding was defined as providing breast milk as the only source of nutrition for the infant. Additional demographic details were provided, including the mothers' education and job status, immigration history of both parents, number of caretakers, parity, and current stress level.

5.4.3 Stimuli

Photographs of three females expressing happiness, fear, anger, and neutrality were chosen from a published and validated stimulus set (FACES Collection) (Ebner, Riediger, & Lindenberger, 2010). The selection was based on an average recognition rate of at least 90% from adult raters across all emotional expressions. Furthermore, we excluded actresses whose hair obstructed the face in any way. Stimuli were created such that each emotional face was presented side-by-side with the neutral face of the same actress. Using Adobe Photoshop (Version CS5), facial expressions were placed within two predetermined ovals within a light gray frame (always located in the same positions, at the same distance and aligned with each other) (Figure 5.2) that removed potentially distracting outer features of the faces such as the ears and parts of the hair. The facial expressions were placed within (below) this frame and were moved and resized to align with geometrically fixed markers for the position of the two eyes, the nose, and the mouth. This editing procedure ensured that all facial stimuli were presented in the same position, all facial features were aligned, and the eyes were within the designated region of interest (ROI) created in Tobii Studio. Facial ovals were 23 cm high and 16.5 cm wide and presented on a 32 × 52-cm (24-inch diagonal) computer monitor placed about 60 cm in front of the infant. Every trial consisted of the presentation of two faces shown side-by-side: one neutral face with no emotional content, and one expression displaying either happiness, fear, or anger. The two faces displayed simultaneously during each trial were aligned and presented 25 cm apart from each other (nose-to-nose distance). The presentation of an emotional face and a neutral face simultaneously allowed for the direct comparison of infants' looking preference for emotional faces compared with neutral faces (Figures 5.1 and 5.2). Information on the calculation of a preference score is located in *Section 5.4.6: Data Analysis*.

A three-second video clip of a shaking rattle was used as an attention-getter to draw infants' attention to the center of the screen before each experimental trial. The rattle measured 3

cm × 1.5 cm and was presented in the center of the screen. It was accompanied by three tones of alternating frequencies (ranging from 109 Hz to 262 Hz).

5.4.4 Procedure

Infants were seated on a parent's lap, ~60 cm away from the presentation monitor. The experimental area was separated from the experimenter's control desk with a room divider. The region behind the computer monitor was covered with a black curtain to prevent any possible distractions for the infant. A small plastic ring was provided for each infant to hold during stimulus presentation. A Tobii ×120 eye tracker was set up at the bottom of the computer monitor to record infant looking behavior. Stimuli were presented through Tobii Studio (Version 3.2). Before stimulus presentation, a five-point calibration procedure was administered. At the five calibration locations (four corners and the center of the screen), a moving rattle of the length of 3 cm was shown. The designated area within the rattle stimulus comprised a visual angle of ~4.6°. The rattle was combined with a beeping sound. For successful calibration, infants needed to fixate within this designated fixation area at all five locations. The calibration procedure was repeated for infants that did not successfully fixate. Additional information regarding the infant calibration procedure is available in the Tobii user manual (available for free download at www.tobii.com).

Every infant viewed nine trials, such that each emotion was presented three times and by each actress. Stimulus presentation was pseudorandomized such that no emotion and no actress were displayed twice in a row. Presentation of the emotion on the left or right side was counterbalanced for each infant. Because there were nine trials, half of the infants had one extra trial where the emotional face was presented on the left whereas the other half had one extra trial where the emotional face was presented on the right. Infant behavior was recorded online such that the experimenter could assess attention and had full control over the presentation of each trial. Each trial began with a three-second attention-getter in the center of the screen (the same

moving rattle plus beeping combination used for calibration). Experimental trials were presented for 15 seconds. During that period, infants were able to freely explore the faces and look toward and away from the screen as often as they pleased. The entire eye-tracking session lasted approximately three minutes.

5.4.5 Genotyping

Saliva samples were collected from infants and their mothers at a previous visit (at five months of age). Sponges were used to collect saliva from infants (OG-250 kit; DNA Genotek), and collection tubes were used for mothers (OG-500 kit; DNA Genotek). Infants and mothers had no food or drink within the 30 minutes before collection. Samples were stored at room temperature, and DNA was extracted using the DNA Genotek manual purification protocol. Genotyping of CD38 rs3796863 was performed with a 5'-nuclease assay. Primers and probes were from Applied Biosystems (TaqMan SNP Genotyping Assay). PCR was conducted with HotStarTaq Plus DNA polymerase and Q-solution (Qiagen) in a Bio- Rad C1000 instrument with a CFX96 fluorescence reading module, with the following thermal protocol: enzyme activation, -95 °C for 5 minutes; cycling, -95 °C for 15 seconds, 60 °C for 1 minute, 45 times.

5.4.6 Data analysis

The duration of exclusive breastfeeding (EBF) was negatively skewed in our sample: $Z_{skewness} = -5.84$, $p < 0.001$; $Z_{kurtosis} = 3.12$, $p < 0.01$ ($M = 151.38$ days, $SEM = 5.59$; *median* = 167.40 days). We therefore used a median split to create categorical groups of low and high EBF for further analysis and visualization (low EBF, $M = 118.59$ days, $SEM = 7.53$; high EBF, $M = 190.87$ days, $SEM = 2.15$) (Katz, 2006). Regions of interest (ROIs) were created within Tobii Studio. ROIs comprised the eye regions of the face stimuli used (Figure 5.2). The total looking times for each emotional eye region were extracted for each infant and trial (see Table 5.6 for raw values). We then computed average looking preference scores by calculating the proportion of

looking time to each emotion (anger (A), fear (F), happiness (H)) and its corresponding neutral face (neutral (A), neutral (F), neutral (H)). The looking preference for any given emotion (X) was computed as follows: looking preference (X) = looking time (emotion X)/(looking time (emotion X) + looking time (neutral X)).

We were therefore able to compare the percentages of looking time toward anger, fear, and happiness, while taking into account the time the infant spent looking at neutral stimuli (see Figure 2 for stimulus example). Critically, looking preferences were calculated only on the basis of trials during which the infant had fixated (looked at) both the emotional face and the neutral face at least once. This guideline served as a minimum criterion for a trial to be included in the analysis. Note that infants had 15 seconds to freely explore, scan, and compare between both faces presented simultaneously on the screen. To present two faces side-by-side for this duration is an experimental procedure commonly used to determine visual preferences for facial expressions in infants of this age (see Grossmann, Striano, & Friederici, 2007; Peltola et al., 2009). Of the maximum number of nine trials, infants contributed an average of $M = 8.85$ trials ($SEM = 0.07$; *range* = 4–9). All infants contributed at least one trial per emotion to the analysis, and the great majority of infants ($N = 92$) contributed all nine trials to the analysis. The number of trials included in the analysis did not differ between EBF groups or genotype groups (all p values > 0.05).

The heat maps of absolute looking durations for each infant during each attention-getter/centering stimulus were visually inspected throughout the session. The visual inspection of these heat maps revealed that the infants fixated on the moving rattle during all nine attention-getters, indicating first that there was no drift across the experimental session, and second that infants maintained central looking before the presentation of the facial stimuli.

5.4.7 Questionnaires

Interpersonal Reactivity Index (Davis, 1983a). The Interpersonal Reactivity Index (IRI) evaluates dispositional empathy. This questionnaire has four subscales and was given to mothers to assess the tendency one has to take the point of view of others (PT), the tendency to experience feelings of sympathy and compassion for those less fortunate (EC), the tendency to experience feelings of distress in response to discomfort in others (PD), and the tendency to transpose oneself into fictional situations such as novels or movies (FS).

The Positive and Negative Affect Schedule (Watson et al., 1988). The Positive and Negative Affect Schedule (PANAS) assessed the general mood of the mother over the last 12 months and calculated both a positive (PA) and negative affect (NA) score.

Additional questionnaires. Additional questionnaires were administered to mothers when infants were 5 months of age (about 2 months before the current study) and were explored in analyses reported here. A short form of the Social Support Questionnaire (SSQ6) was filled out by mothers (Sarason, Sarason, Shearin, Pierce, & Pierce, 1987). The SSQ6 comprises two subscales that measure the amount of social support one experiences (SSQ Number), as well as the satisfaction one experiences with the support (SSQ Satisfaction). The Parental Sense of Competence (PSOC) questionnaire assessed how knowledgeable and capable each mother felt with parenting (Johnston & Mash, 1989). Lastly, the Edinburgh Postnatal Depression Questionnaire (EPDS) was administered to each mother to detect potential signs of postpartum depression (Cox, Holden, & Sagovsky, 1987).

Analysis of Questionnaire and Demographic Data. To identify and rule out potentially confounding factors, analyses (one-way ANOVAs) were performed to compare between EBF and genotype groups for the questionnaire and demographic data. These variables included demographic factors such as maternal age and infant age, parity, years of maternal education, number of caretakers, and current subjective stress level of the mother. Additional maternal self-report measures were analyzed, including amount of, and satisfaction with, social support,

parental sense of competence, Edinburgh postnatal depression score, positive and negative affect score, and all subscores on the interpersonal reactivity index (empathic concern, fantasy seeking, personal distress, perspective taking). None of these variables differed significantly between EBF groups or genotype groups (all p-values > 0.1). Moreover, there was no difference between EBF groups or genotype groups with respect to the delivery method (vaginal birth versus caesarean section) ($\chi^2(1) = 1.206$, $p = 0.272$; $\chi^2(1) = 1.037$, $p = 0.309$, respectively). The absence of differences across EBF and genotype groups with respect to these variables helps to rule out that any of those variables account for the effects reported in the current study.

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5.5 Supporting Information

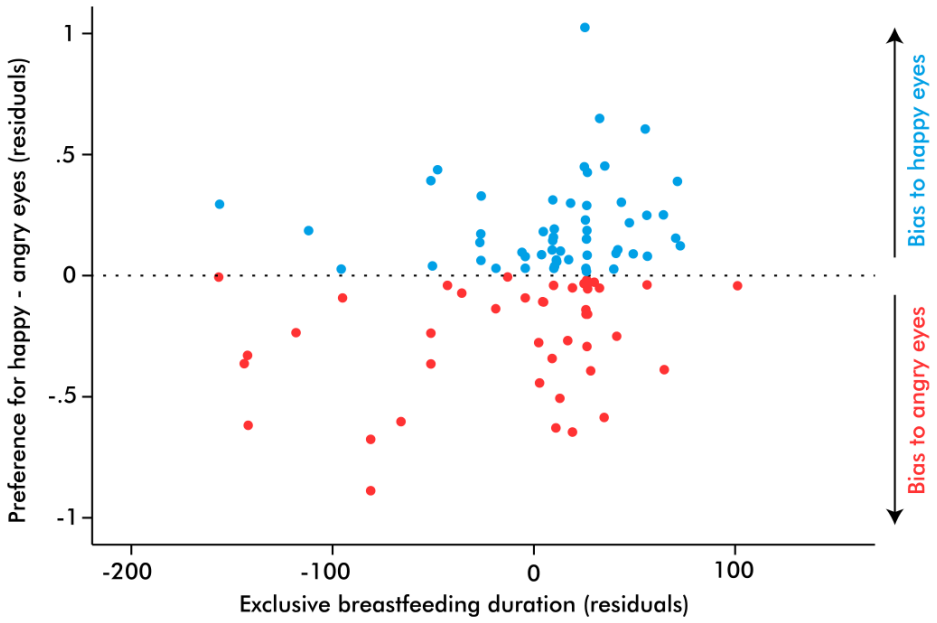

Figure 5.5 (S1) Partial regression plot illustrating the prediction of EBF on infant looking preference to happy eyes minus angry eyes. Please note that the residuals from the regression are plotted on the x and y axes. Points above zero represent a bias toward happy stimuli whereas points below zero represent a bias toward anger. $p = 0.006$, $N = 98$.

Table 5.4 (S1) Maternal *CD38* rs3796863 genotype frequencies.

Genotype	Frequency	%
CC	60	61.2
CA	29	29.6
AA	9	9.2

Note that the maternal genotypes were used to conduct Mendelian inheritance analyses and are shown only for the sake of completeness. They were not used in any of the main analyses of this study.

Table 5.5 (S2) Descriptive statistics for main breastfeeding variables and covariates maternal age and education split by genotype and breastfeeding groups.

	CC		CA/AA	
Variable	EBF low	EBF high	EBF low	EBF high
EBF duration, d	115.52 (12.77)	193.55 (3.69)	120.77 (9.31)	188.42 (2.31)
Currently breastfed meals, %	38.32 (8.16)	71.64 (4.02)	35.36 (5.79)	63.61 (5.53)
Maternal age, y	31.45 (1.03)	30.86 (0.86)	31.00 (0.64)	32.78 (1.13)
Maternal education, y	16.55 (0.83)	16.82 (0.54)	15.87 (0.59)	17.39 (3.81)

Values are presented as mean (SEM).

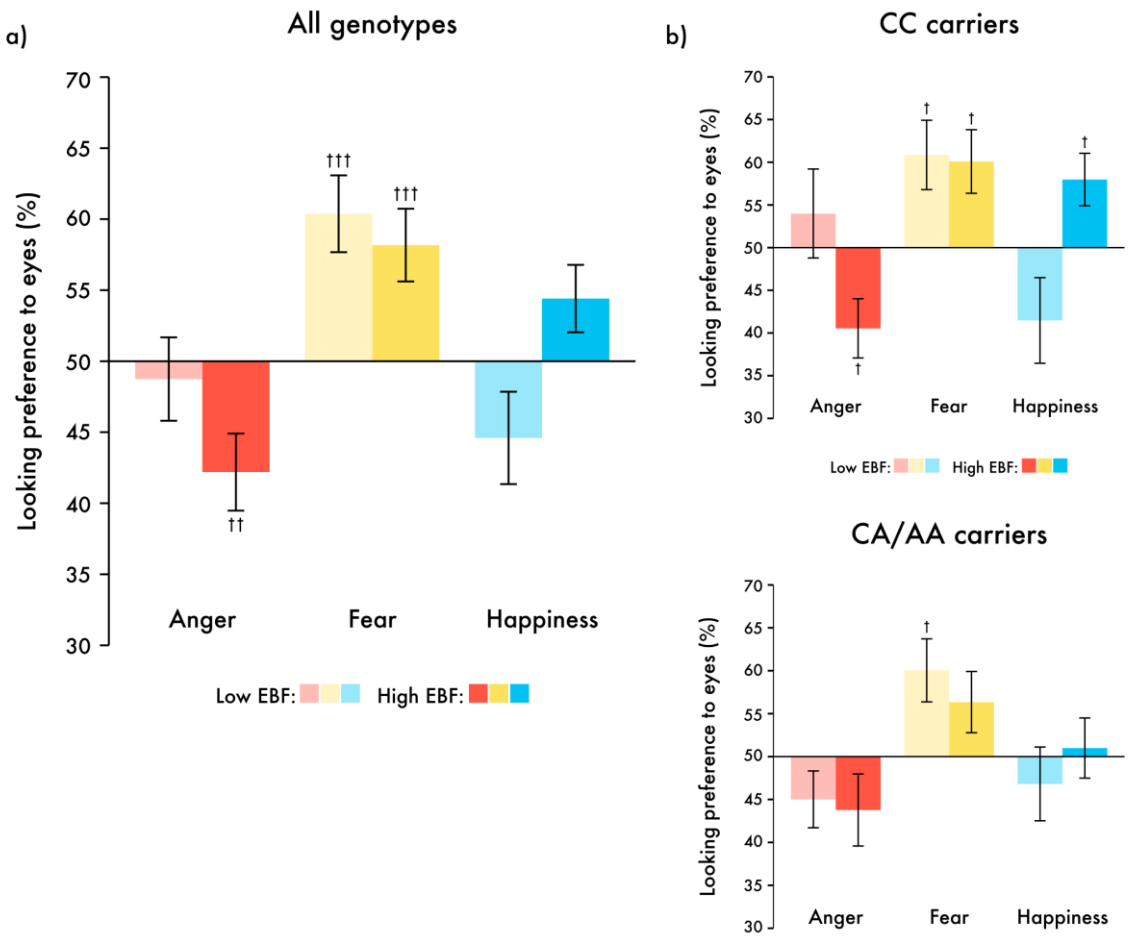

Figure 5.6 (S2) Bar graphs illustrating the results of one-sample *t* tests in which we compared each looking preference score to chance (50%) when (A) split by EBF group and (B) split by EBF and genotype groups. $^{\dagger}p < 0.05$, $^{\dagger\dagger}p < 0.01$, and $^{\dagger\dagger\dagger}p < 0.001$.

Table 5.6 (S3) Raw looking durations (seconds) for each emotion, split by genotype and breastfeeding groups.

	CC		CA/AA	
Emotion	EBF low	EBF high	EBF low	EBF high
Angry eyes	3.93 (0.54)	4.00 (0.57)	4.61 (0.62)	5.08 (0.79)
Fearful eyes	5.56 (0.94)	5.72 (0.75)	6.89 (0.97)	8.00 (1.06)
Happy eyes	3.45 (0.60)	4.85 (0.60)	4.21 (0.62)	6.10 (0.92)

Values are presented as mean (SEM). Total looking durations did not differ significantly between EBF or CD38 groups.

Part III. Discussion

Chapter 6.
General discussion and outlook

6.1 Summary of empirical studies

The current thesis investigated how individual differences related to the oxytocin system impact emotion perception in mothers and infants. Specifically, I examined breastfeeding, a naturally occurring behavior in mothers and infants with strong ties to the oxytocin system, as well as genetic differences within the oxytocin system itself. Through this, I systematically examined how exogenous and endogenous oxytocin exposure might lead to tangible emotional biases. In line with oxytocin administration studies in adults, I had hypothesized that an extended exposure to breastfeeding might facilitate the processing of happy emotional expressions, and perhaps, attenuate processing of negative emotional expressions. For the purposes of this research, a variety of methodologies were used. *Study 1* aimed to most closely replicate adult intranasal oxytocin studies by using exclusive breastfeeding experience as a proxy for exogenous oxytocin levels. A dynamic emotional expression task was administered to mothers of five- to seven-month-old infants and reaction times to dynamic emotional expressions were examined. In *Study 2*, using ERPs, the neural processing of happy and fearful body expressions was examined in relation to exclusive breastfeeding experience in eight-month-old infants. In *Study 3*, infant looking preferences to the eye region of happy, fearful, and angry faces were assessed and related to both breastfeeding experience and genetic differences in the oxytocin system (*CD38*). Thus, the current thesis explored the impact of breastfeeding on emotion perception in a variety of contexts, including age (infancy, motherhood), expression domain (bodies, faces, eyes), and genetics (single-nucleotide polymorphism of the CD38 gene).

The results from *Study 1* indicate that a longer duration of exclusive breastfeeding is associated with quicker reaction times to happy facial expressions in mothers. In contrast, a greater percentage of breastfed meals slowed down the reaction times to angry expressions. Critically, these results could not be explained by potentially confounding variables, including maternal education, mood, parity, or infant temperament. This pattern of results is in agreement with oxytocin administration studies in which oxytocin increases the salience of positive social cues and attenuates salience of negative cues (Domes, Steiner, et al., 2013; Kirsch et al., 2005; Marsh et al., 2010), and more generally, these findings inform the substantial body of evidence relating oxytocin to prosocial behavior (Kanat, Heinrichs, & Domes, 2014). Taken together, *Study 1* identifies breastfeeding as a natural context during which the social effects of oxytocin can be observed. Findings also support evolutionary theories of which maternal behavior is tuned to prosocial responsiveness and bonding, with its vital elements facilitating the rapid and accurate responding to affiliative cues (Feldman, 2012c; Hrdy, 2009).

While *Study 1* found an impact of breastfeeding on emotion perception in mothers, *Study 2* examined whether this impact might extend to infants. For this purpose, the Nc in response to emotional body expressions was assessed in eight-month-old infants. The Nc is an index of attentional allocation in infants (de Haan, 2007b) (see Chapter 2.2). In this study, the size of the Nc to happy and fearful body expressions interacted with exclusive breastfeeding experience. Specifically, the neural response to happy body expressions was greater in infants with high levels of exclusive breastfeeding as compared to infants with low exclusive breastfeeding experience. Thus, more exclusive breastfeeding led to a greater attention to happiness. The impact of breastfeeding duration was linear, such that as exclusive breastfeeding increased, an attentional bias towards fearful bodies shifted to one towards happy bodies. Again, a variety of confounding variables were accounted for, including maternal education and percentage of currently breastfed meals. These results are in line with *Study 1*, suggesting that breastfeeding experience increases the salience of prosocial stimuli by decreasing the salience of negative, threatening stimuli. They

are also in agreement with adult oxytocin administration studies, suggesting again that breastfeeding may be a natural context in which the prosocial effects of oxytocin can be observed. Importantly, this study provides first evidence that breastfeeding may be a proxy for oxytocin levels in human infants, and thus, can critically impact emotion processing at an early age. More generally, such an increased sensitivity to positive emotional signals may be important in fostering positive social interactions and thereby may serve important affiliative functions in human development.

Study 3 had several research aims. One aim was to investigate whether the impact of breastfeeding on attention to emotional body expressions in infants could be extended to attention to the eye region of facial expressions, and another aim was to find out whether genetic differences within the oxytocin system interact with breastfeeding to modulate attention to emotions. For this purpose, seven-month-old infants were presented with two faces simultaneously: one neutral, one emotional (happy, fearful or angry) and looking preferences were assessed. Our results demonstrate that, in agreement with prior work, infants at this age show a robust fear bias (Peltola et al., 2013). That is, seven-month-old infants looked longer at the eye region of fearful faces as compared to the eye region of neutral faces. Furthermore, our data revealed an interaction between emotion and exclusive breastfeeding such that high exclusively breastfed infants looked at happy eyes more than low exclusively breastfed infants. These results further supported our hypotheses and findings from *Studies 1* and *2*, signifying a positive bias with increased exclusive breastfeeding. Lastly, and most noteworthy, this interaction was modulated by *CD38* genotype. The impact of exclusive breastfeeding on emotional eye preference was only significant in infants with the "risk" genotype (CC); that is, the genotype associated with increased risk for autism and lower plasma oxytocin in general. Within this genotype, infants with the highest duration of exclusive breastfeeding showed a greater preference for happy eyes and a lower preference for angry eyes than infants with the lowest exclusive breastfeeding duration. This suggests that infants genetically at risk for autism are

impacted most in their emotion processing when exclusively breastfed for longer durations. Importantly, these findings could not be explained by maternal education, age, or currently breastfed meals.

In summary, the current thesis provides first evidence that breastfeeding exposure, and particularly *exclusive* breastfeeding duration, is associated with individual differences in emotion processing in both mothers and infants. Specifically, the results presented in the current thesis show that an extended duration of exclusive breastfeeding increases the salience of positive, prosocial and affiliative cues, and reduces the salience of negative, antisocial and interpersonally threatening cues.

The findings from this thesis are novel in several ways. Firstly, while there is much research and great agreement on the health and cognitive benefits of exclusive breastfeeding, these are the first studies to investigate the influence of breastfeeding on aspects of social functioning and development. Breastfeeding is an intimate, social behavior shared by both mothers and infants. By studying effects of breastfeeding in both mothers and infants, I have considered the mother and infant as a dyadic system. I examined emotion perception using several types of stimuli, including expressions from bodies, dynamic faces, and eye regions alone. I measured perception using behavioral indices such as reaction time and looking preference, as well as neural measures of attention using ERPs. By investigating both breastfeeding exposure and genetic differences within the oxytocin system, I have researched how nature and nurture interact to impact emotional processing in a holistic manner. These findings present a fresh perspective on the function of breastfeeding. While it is undoubtedly important to provide nutrition to the infant, the work presented herein suggests that it also crucially impacts social and emotional functioning in both mothers and infants. This is arguably due to its strong relationship to the oxytocin system. The present findings are in agreement with a wealth of studies regarding the function of the oxytocin system, which highlight its crucial role in prosocial and affiliative behavior (Feldman, 2012b; Lee, Macbeth, Pagani, & Young, 2009). By facilitating the salience of

positive emotional expressions, breastfeeding may act in a similar manner by predisposing one to become more tuned to prosocial signals. Moreover, findings contribute to a wealth of animal and human research which has demonstrated the impact early experience of maternal care can have on early social development (Champagne & Curley, 2009; Gee et al., 2013).

6.2 Implications for future work

The results of this thesis have particular implications when considering how emotional biases develop and impact daily life. The ability to read and make meaning out of others' expressions allows us to predict future behavior and plan our own behavior accordingly. Not unlike most human capabilities, there is considerable variation between individuals in emotional processing. For example, atypical patterns of emotion perception and recognition are reported in a range of mental health disorders, including depression, social phobia, schizophrenia, borderline personality disorder, and autism spectrum disorders (ASD) (Aldinger et al., 2013; Baron-Cohen, 1995; Baron-Cohen, Wheelwright, Hill, Raste, & Plumb, 2001; Demenescu, Kortekaas, den Boer, & Aleman, 2010; Domes, Schulze, & Herpertz, 2009; Kohler, Bilker, Hagendoorn, Gur, & Gur, 2000). For example, Aaron Beck's cognitive model of depression posits that depressed individuals hold negative biases of themselves, their current experiences, as well as negative projections about their future (Beck, 1976). This negative bias has been a target of both cognitive therapy (to remediate these biases) and antidepressants (Harmer, Goodwin, & Cowen, 2009; Roiser et al., 2012). Patients with major depression tend to have an enhanced memory for negative material in explicit memory paradigms (Leppänen, 2006), failure to disengage attention from negative stimuli (Mogg, Bradley, & Williams, 1995), and a tendency to label ambiguous expressions as negative (i.e. facial expressions, Gur et al., 1992). It is unknown whether these biases are manifestations of the disease itself, or whether those with negative biases may be more predisposed to develop depression (Harmer et al., 2009).

For mothers, our research might have important implications with respect to postpartum depression. Previous literature suggests that breastfeeding may be a protective factor for mothers who are at risk for developing depression due to its anti-inflammatory and anti-stress effects (Kendall-Tackett, 2007). There is also evidence to suggest that some mothers develop depressive symptoms post-weaning (cessation of breastfeeding) (Susman & Katz, 1988). Our research may extend and inform these hypotheses by highlighting that breastfeeding has more far-reaching effects on how emotional information is processed more generally. Thus, based on the results of *Study 1*, it is possible that longer durations of exclusive breastfeeding may reduce emotional biases related to depressive symptoms during the postpartum period. Future research is needed to examine this possibility and gain a more mechanistic understanding of the effects of breastfeeding on mothers' emotion processing. This may ultimately inform preventative measures for mothers at risk for postpartum depression.

Another important issue that warrants discussion is whether the emotional processing biases found as a function of breastfeeding continue to persist beyond weaning into childhood and adolescence. We know from animal research that subtle differences in maternal care can alter the brains and behavior of the young (please see Chapter 1.3.1). For example, not only can the amount of licking and grooming a rat dam provides to her offspring dramatically change the stress reactivity of her offspring by altering receptor frequencies, but female pups who receive high maternal care will exhibit high maternal care to their own offspring (Champagne et al., 2003; Liu et al., 1997). It is thus possible that different durations of breastfeeding can have a long-term impact on the way emotions are processed. Along these lines, it will be important to elucidate whether the emotional processing biases seen in infants in the current studies translate into differences in overt social behavior. For example, increased attention towards happiness and decreased attention to anger may increase approach and social behaviors such as helping, sharing, and comforting. Moreover, the current studies may have particular relevance for infants at risk for ASD. As shown in *Study 3*, only infants with a particular genotype associated with lower

oxytocin levels and greater risk for autism (*CD38* rs3796863 CC) were impacted by exclusive breastfeeding duration: these infants showed greater attention to happiness and reduced attention to anger with longer breastfeeding experience. There is the possibility that this emotion processing bias observed in infancy translates into overt social behavioral effects in childhood. If so, breastfeeding experience may help facilitate prosocial tendencies in children genetically at risk for autism. In order to examine this question we are currently following the cohort from *Study 3* in a longitudinal study design in which we will assess their social behaviors, including helping, sharing, and comforting in the second year of life.

Our findings regarding the *CD38* genotype may also have implications for oxytocin administration studies. Currently, there are several clinical trials attempting to use oxytocin as a treatment for autism (clinicaltrials.gov). Completed trials have generally provided mixed results (Anagnostou et al., 2014; Anagnostou et al., 2012; Bakermans-Kranenburg & van IJzendoorn, 2013). Our findings from *Study 3* indicate that genetic variation impacting endogenous oxytocin levels influence how one responds to exogenous stimulation of the oxytocin system. It is possible that a person's ability to release oxytocin impacts how they will respond to administration of intranasal oxytocin. An approach in which factors influencing the endogenous oxytocin system are assessed may help to identify individuals who will be most responsive to oxytocin administration treatment.

6.3 Limitations and future directions

The current thesis investigated a novel and important question: Does breastfeeding impact social and emotional functioning of mothers and infants? As with all novel research, the findings presented in this thesis must be interpreted with caution and should only be seen as a first step at answering this complex question. There are several limitations that require attention and thus, may in fact open up new avenues for future research.

First and foremost, breastfeeding is a highly complex maternal behavior. In the current thesis we have attempted to study the effects of this highly complex behavior by taking into account (and controlling for) other factors that may impact breastfeeding behavior such as maternal sense of competence, social support, education, general mood, empathy, as well as aspects of infant temperament. However, one aspect that may need further attention is more specifically related to the decision to breastfeed and the determination with which breastfeeding is continued. These decision processes, which were not evaluated in our research, may well have had an impact on the duration of exclusive breastfeeding examined in the current studies. Furthermore, the general aim of this thesis was to investigate breastfeeding exposure as a proxy for oxytocin levels. However, oxytocin levels were not directly tested in our samples, and thus, until directly tested, oxytocin must only be considered a potential pathway that can account for our results. Moreover, while breastfeeding is linked to elevated oxytocin in mothers (Dawood et al., 1981), whether oxytocin levels rise in human infants remains unknown. For now, we can only infer that oxytocin levels rise in human infants because research with other mammals suggests that this is the case (Lupoli et al., 2001; Takeda et al., 1986).

Breastfeeding itself consists of other factors that not only may affect oxytocin levels on their own, but may impact emotion perception. For example, skin-to-skin contact has been found to influence emotion regulation, attention, joint engagement, and pain analgesia (Feldman et al., 2014; Gray et al., 2000). Breastfeeding also impacts the HPA system and cardiovascular activity of mothers; and perhaps that of infants as well (Heinrichs et al., 2002) (see Chapter 1.3.2). These mechanisms alone could impact how mothers and infants perceive emotional expressions (Douglas & Porter, 2012; Ellenbogen, Schwartzman, Stewart, & Walker, 2002).

Another important limitation is that breastfeeding is a naturally occurring behavior strictly determined by the mothers. While this should not be seen as a limitation per se, it meant that I could not randomly assign mothers to different breastfeeding groups, as this would be unethical. I therefore tried to assess and quantify breastfeeding behavior as carefully and systematically as

possible (i.e. duration of exclusive breastfeeding, daily percentage of breastfed meals). One potential avenue for future work would be to investigate emotion perception in both bottle-feeding and breastfeeding infants and mothers. For example, one could directly compare duration of "exclusive" bottle-feeding with duration of exclusive breastfeeding. For the purposes of this thesis I was most interested in variance *within* breastfeeding dyads, due to the established connection to the oxytocin system. However, it could be possible that the social elements of bottle-feeding lead to changes within the oxytocin system (or others) as well. It will be important to explore these ideas in future research, and of course, to replicate and extend the findings of the current thesis in more socio-economically diverse samples.

Future research into the mechanisms of breastfeeding, particularly within the infant, will be crucial for the interpretation of the current findings. For example, if the emotion processing biases seen in infants are indeed linked to the breast milk itself, then one possibility might be to encourage and support the use of natural breast milk from breast milk banks for those who cannot breastfeed on their own. Alternatively, a better understanding of the mechanisms and effects may inform novel developments in the production of formula milk (i.e., the addition of LCPUFAs to promote neural growth). It is also possible that the emotional biases are an outcome of the skin-to-skin contact or warmth that is characteristic of breastfeeding. If this is the case, it may encourage parents to engage in more situations with warm skin contact, such as the already established "Kangaroo Care", often used with preterm infants (Head, 2014; McGregor & Casey, 2012). As mentioned above, longitudinal studies are greatly needed to investigate whether effects seen in infants persist into childhood. For now, we have no evidence to suggest that a bias toward happiness in infancy is any more beneficial than a bias toward anger or fear. We can only speculate at this point, and it will be important to follow developmental trajectories beyond the first year of life.

As a final future direction, I believe the field of epigenetics will be especially important for this research. As mentioned in the introduction, certain elements of maternal care in animals

can alter gene expression in offspring (Champagne & Curley, 2009). This is due to DNA methylation, in which certain sections of our predetermined DNA can be rendered more or less productive by the amount of methyl groups present (Reik, 2007). Methylation occurs in all of us, and is something completely driven by experience. It may prove to be an interesting mechanism through which exclusive breastfeeding exerts its effects in humans. For example, it could be possible that the duration of exclusive breastfeeding critically impacts the oxytocin systems of infants by either increasing or decreasing methylation of the oxytocin gene (*OXT*), its receptor gene (*OXTR*), or associated genes (i.e., *CD38*). We are currently investigating this very question with follow-up studies on the cohort of *Study 3*.

In conclusion, the current thesis served as a first step to investigate whether and how breastfeeding impacts emotion perception in mothers and infants. Taken together, the present findings contribute to a more comprehensive understanding of the underlying factors that contribute to individual differences in the perception of emotions. The combined evidence from this thesis provides a highly novel contribution, suggesting that emotion perception is flexible and likely to be susceptible to aspects of the external (breastfeeding) and internal (*CD38* genotype) environment. It is my hope that the findings from this thesis encourage future work in this field. This in turn will facilitate a more integrated understanding of how social development unfolds as an interplay between nature and nurture.

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Erklärung / Declaration

Erklärung gemäß § 8 Abs. 1 Buchst. b) und c) der Promotionsordnung der Fakultät für Verhaltens- und Empirische Kulturwissenschaften
Declaration in accordance to § 8 (1) b) and § 8 (1) c) of the doctoral degree regulation of Heidelberg University, Faculty of Behavioural and Cultural Studies

Promotionsausschuss der Fakultät für Verhaltens- und Empirische Kulturwissenschaften der Ruprecht-Karls-Universität Heidelberg
Doctoral Committee of the Faculty of Behavioural and Cultural Studies of Heidelberg University

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