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Early Communication: The Role of Oxytocin in Sharing Emotions with Mother during the First Year of Life

Raná komunikace: Role oxytocinu v sdílení emocí s matkou během prvního roku života

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Prohlašuji, že jsem diplomovou práci vypracovala samostatně, že jsem řádně citovala všechny použité prameny a literaturu a že práce nebyla využita v rámci jiného vysokoškolského studia či k získání jiného nebo stejného titulu.

V Praze 12. 4. 2013

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Abstrakt

Nedávné studie naznačují, že oxytocin hraje významnou roli v interakci matky s dítětem. Předkládaný výzkum se zaměřil na probádání vztahu mezi Mateřským emocionálním sladěním (Maternal Affect Attunement) a hladinami oxytocinu u matek a jejich dětí během rané komunikace a na prozkoumání vztahu hladiny oxytocinu matek a jejich dětí. Studie se účastnilo 43 párů matka-dítě, přičemž dětem byly 4 měsíce. Pozorování těchto párů matka-dítě probíhalo za tří různých podmínek: 1) Základní – kdy neprobíhala žádná komunikace. 2) Přirozená interakce – kdy matka s dítětem komunikovala přirozeně. 3) Modifikované interakce – během nichž byla přirozená interakce mezi matkou a dítětem různými způsoby narušena. Během výzkumné procedury byly ve čtyři různé časové body odebrány vzorky slin matek a dětí, z nichž byla poté zjišťována hladina oxytocinu. K vyhodnocení stylu interakce matky bylo během Přirozené interakce kódováno Mateřské emocionální sladění (škály Udržování pozornosti (Maintaining Attention), Vřelá citlivost (Warm Sensitivity)). Výsledky ukázaly, že hladiny oxytocinu matky a dítěte byly stabilní ve všech čtyřech časových bodech, kdy byly vzorky odebírány. Hladiny oxytocinu matky ve všech bodech odběru, stejně tak jako celková průměrná hladina oxytocinu, negativně korelovaly s Vřelou citlivostí. Byly nalezeny jak signifikantní pozitivní korelace mezi hladinou oxytocinu matky a dítěte u odběrů ve stejný čas, tak signifikantní pozitivní opožděné korelace (cross-lagged correlation). Tyto výsledky naznačují, že hladina oxytocinu matek měřená ze slin může odrážet individuální rozdíly v jejich Vřelé citlivosti a dále předkládají další důkazy o tom, že oxytocinový systém matek a jejich dětí je propojen. Výsledky této studie tedy poukazují na důležitost studování neurobiologických korelátů interakce matky-dítě.

Klíčová slova

komunikace mezi matkou a dítětem, oxytocin, sdílení emocí, mateřské emocionální sladění

Abstract

Recent studies support the hypothesis that oxytocin plays an important role in mother-infant interactions. The aim of the present research was to investigate the connection between Maternal Affect Attunement and levels of oxytocin in mothers and infants during early mother-infant communication, and to explore the relationship between maternal and infants' oxytocin levels. Forty three mother-infant dyads participated in the present study when infants were four months. They were observed in three conditions: 1) Baseline – where no communication took place, 2) Natural Interaction between mother and infant and 3) Modified Interactions - where natural interaction between mothers and infants was disrupted in various ways. During this procedure four saliva samples from mothers and their infants were collected to determine their levels of oxytocin at different time points. To assess individual maternal interactive style Maternal Affect Attunement (Maintaining Attention, Warm Sensitivity) was coded during the Natural Interaction. Results indicated that maternal and infants' oxytocin levels were stable at the four collection points. Maternal oxytocin levels at all collection points as well as overall mean oxytocin level were negatively related to her Warm Sensitivity. Significant positive synchronized as well as cross-lagged correlations were found between maternal and infant oxytocin levels. These results suggest that maternal salivary oxytocin may reflect individual differences in the degree of her Warm Sensitivity, and demonstrate further evidence that maternal and infants' oxytocin functioning is interconnected. Findings of the present study thus show the importance of examining the neurobiological correlates of mother-infant interaction.

Keywords

mother infant communication, oxytocin, emotion sharing, Maternal Affect Attunement

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List of Acronyms

ACC	Anterior cingulate cortex
AMG	Amygdala
ANCOVA	Analysis of Covariance
BDI-II	Beck Depression Inventory
EIA kit	Enzym Immuno Assay Kit
HPA axis	Hypothalamic-pituitary-adrenal axis
MFAS	Maternal-Fetal Attachment Scale
MPFC	Medial prefrontal cortex
MRI	Magnetic Resonance Imaging
NAS	Nucleus accumbens
OFC	Orbitofrontal cortex
OXT	Oxytocin
PVN	Paraventricular nucleus
SN	Substantia nigra
SON	Supraoptic nucleus
STR	Striatum
VMH	Ventromedial hypothalamus
VTA	Ventral tegmental area

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1. Introduction

Early mother-infant interactions have an impact on the infant's social, emotional and cognitive development (Bornstein, 2002; Bowlby, 1969; Schore, 2001a; Stams, Juffer, & van IJzendoorn, 2002). Therefore, it is necessary to study the main factors influencing its quality. Animal research has shown that the neurohormone oxytocin plays a crucial role in maternal behavior and influences mother-infant interactions (Carter, 1998; Keverne & Kendrick, 1992; Lim & Young, 2006; Maestripieri, Hoffman, Anderson, Carter, & Higley, 2009; Maestripieri, 1999; Ross et al., 2009). In humans, biology is not unavoidably destined since we have the ability to make choices (Panksepp, 2004), but recent human studies similarly suggest that oxytocin also plays an important role in mother-infant interaction (Bakermans-Kranenburg & van IJzendoorn, 2008; Feldman, Gordon, & Zagoory-Sharon, 2010, 2011; Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010; for review, see Galbally, Lewis, van IJzendoorn, & Permezel, 2011). Specifically, higher maternal oxytocin measured in the first trimester of pregnancy and in the first postpartum months predicted the amount of maternal behaviors, such as gazing at infants, 'motherese' vocalizations, positive affect and affectionate touch (Feldman et al., 2007). These maternal behaviors communicate the mother's interest for infants and help the mother-infant dyad to develop trust and to bond (Bornstein, 2002; Papoušek & Papoušek, 1995). Moreover, they predict infants' social, emotional and cognitive competencies in early childhood (Feldman & Eidelman, 2009). The level of oxytocin increased in mothers and their infants following face-to-face interaction, and mothers' and infants' oxytocin levels were correlated both pre– and post– interaction suggesting that oxytocin systems of mother and infant are interdependent (Feldman, Gordon, & Zagoory-Sharon, 2010).

There are different approaches to studying mother-infant interaction. For example, Feldman and colleagues (2010; 2011) used the concept of

synchrony, which considers time as a central parameter for studying mother-infant interaction. Importantly, Feldman and colleagues (2010) showed that the degree of affect synchrony between parents and infants in a face-to-face interaction moderated the relation between levels of oxytocin in parents and infants. That is, although infant levels of oxytocin were independently predicted by parental oxytocin, under conditions of high levels of affect synchrony infants whose parents had high oxytocin levels had a significantly higher oxytocin level than infants whose parents had relatively low oxytocin levels. These differences vanished under conditions of low affect - no differences were found in infants' levels of oxytocin among infants whose parents had high or low level of oxytocin (Feldman, Gordon, & Zagoory-Sharon, 2010). These results demonstrated that a closer link was found between parents' and infants' oxytocin levels among parent-infant dyads under conditions of high affect synchrony. Thus, parent-infant synchrony may be the one mechanism by which the functioning of the oxytocin system is transmitted from parents to infants (Feldman, Gordon, & Zagoory-Sharon, 2010).

An alternative promising research approach in mother-infant interaction focuses on the importance of sharing emotions in mother-infant communication (Markova & Legerstee, 2006; Stern, 1984; Trevarthen & Aitken, 2001). This concept considers emotion as a central parameter for studying mother-infant interaction. According to this view, shared emotions are the main components in mother-infant communication and infants from the early beginning of life are able to share their own emotions with others (Legerstee, 2005; Sroufe, 1997). Moreover, it has been found that sharing emotions between mother and infant is influenced by Maternal Affect Attunement (Landry, Smith, Miller-Loncar, & Swank, 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). Maternal Affect Attunement is characterized by maternal interactive behaviors including following or maintaining the infant's focus of interest, expressing positive affect and acceptance of the infant's interest throughout the interaction, using 'motherese' vocalizations, showing concern for comfort, prompt responding

to the infant's signals as well as modulation of any negative infant behavior (Bartling, Kopp, & Lindenberger, 2010; Legerstee et al., 2007; Markova & Legerstee, 2006). In other words, highly attuned mothers express the quality of a shared affect state without imitating the exact overt behavior of the inner state (Stern, 1985). Maternal Affect Attunement has been shown to be important for infants to learn interaction skills such as taking an active role in communication, responsiveness to the mother's signal, ability to progress from dyadic interaction to triadic interaction and ability to differentiate between different interactive conditions (Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). Because Maternal Affect Attunement is associated with early communicative exchanges between mothers and infants, it would be interesting to observe the relationship between oxytocin functioning in mother-infant dyads and Maternal Affect Attunement.

However, to date, no study has addressed this relationship. Thus, the first aim of the present study was to investigate the relationship between levels of oxytocin in mothers and infants during mother-infant communication and Maternal Affect Attunement. Understanding such links is important for uncovering the biological basis of sharing emotions between mothers and infants and may help to shed more light on understanding the oxytocin system. Although research on the functions of oxytocin is still in its preliminary stages, potential implications of findings for practice could include answers to questions such as: Is it possible that suboptimal oxytocin functioning could be one of the causes for dysfunctional mother-infant interaction? What are the best possible treatment strategies for mothers who have difficulties sharing emotions with their infants? Could medical treatment regulating maternal levels of oxytocin be helpful? What are the best strategies for helping the mother-infant dyad to change dysfunctional aspects of sharing emotions and reestablishing a healthy mutual relationship?

The second aim of the present study was to extend previous findings concerning the interdependency of maternal and infants' oxytocin functioning (Feldman, Gordon, & Zagoory-Sharon, 2010). Early mother-

infant interactions are a mutual and bidirectional process and it has been suggested that during the attuned interactions, mothers and infants coordinate not only behaviors, but also physiological rhythms (Field, 1985). Therefore, oxytocin levels in mothers were expected to correlate with infant oxytocin levels at all points of measurement.

The third aim of the present study was to broaden findings concerning the timing of sampling used in the research using salivary samples to measure levels of oxytocin in mother-infant interaction. Although oxytocin measured in saliva was shown to be a valid biomarker (Carter et al., 2007), there is still no standardized protocol for collecting saliva in order to measure changes in oxytocin levels. Even though it seems to be standard for psychoneuroendocrinological studies to measure oxytocin levels at the beginning of mother-infant interaction and then 15 minutes after the end of interaction (Feldman, Gordon, & Zagoory-Sharon, 2010; Feldman et al., 2011), there is very little data on the reactivity of oxytocin in different time periods. Thus, in this study we collected four consecutive salivary samples taken from both mothers and infants.

2. Mother-Infant Interaction

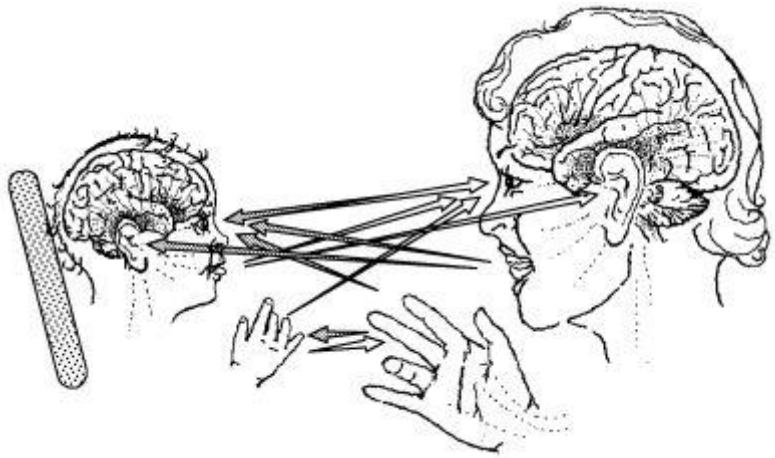
The process of early interaction between mothers and infants is characterized by face-to-face context, close physical contact and its turn-taking structure, where cycles of mutual attention between mothers and infants (social engagement) and cycles of nonattention (disengagement) alternate (Brazelton, Koslowski, & Main, 1974; Field, 1978; Papoušek & Papoušek, 1995; Stern, 1985; Trevarthen, 1993). There is a consensus between developmental psychologists when it comes to describing mother-infant interactions. However, there is a lack of agreement concerning the age of the onset of infants' active role in communication, infants' awareness of mental states and their ability to share meaning and emotions with others (Legerstee, 2009). The theoretical basis of the present study originates in the concepts of continuous developmentalists who argue that even newborn infants are intrinsically motivated to communicate, they act as an active communication partner, and that from the early beginning of life they share emotions with others (Fogel, 1993; Legerstee, 2005; Sroufe, 1997; Stern, 1984; Trevarthen, 1993; Trevarthen & Aitken, 2001).

Thus, early interactions can be described as a dialogue or a mutual, bidirectional process (Tronick, 1989), in which both partners modulate the timing, the form and the intensity of interaction and their own emotional expression to achieve complementary interactive exchanges (Ammaniti & Trentini, 2009). In these exchanges, mothers and infants communicate their own experience to their communicative partner. Even from the early months of life infants begin to communicate on a pre-verbal level and both mothers and infants are well equipped for these pre-verbal communication exchanges (Murray & Trevarthen, 1986; Trevarthen, 2005). For example, human eyes appear to be evolutionarily adapted for the communication of thinking states (Kobayashi & Kohshima, 2001). Also, the maternal emotionally expressive face is the most potent visual stimulus in the infants'

environment, and infants' intense interest in their mother's face and her eyes leads them to track it in space and to engage in mutual gazing (Schore, 2001b). Hands are also adapted to share intentions and feelings (Trevarthen, 1986) and the hands of infants can express subtle changes in alertness, directions of interest, and confident or hesitant purposes (Trevarthen, 2005). Finally, infants are sensitive to and respond to speech patterns from birth and their movements are synchronized to the pace and intensity of speech sound (Condon & Sander, 1974).

Thus, mothers and infants share meanings, intentions and emotions through vocalizing, facial expressions, gazing, touching and gestures (see Figure 1), suggesting that mother-infant communication often takes place across different modalities (Stern, 1985; Trevarthen, 1993). That is, the modality of expression used by mothers as a response to infant expressions may be different from the modality used by infants. For example, an infant would often express happiness with a smile and her mother could respond to it verbally ("You do like this toy, don't you?"), by smiling, or she could touch the infant and initiate play.

Figure 1. Channels Active in Mother-Infant Face-to-Face Communication (Trevarthen & Aitken, 2001).



Eye-to-eye orientation, vocal expressions, hand gestures and head movements act in coordination to express and share emotions.

Although sharing meanings, intentions and emotions through vocalizing, facial expressions, gazing, touching and gestures seems to be nearly universal, the quality of mother-infant interaction is a subject to large variations across the population (Sroufe, 2005). Several factors influencing the quality of mother-infant interactions have been described. Some of them are maternal characteristics (e.g., age, maternal interactive style, prior mothering experience, mother's work experiences), infant characteristics (e.g., temperament, negative affect, irritability) and culture (Belsky, 1984; Bornstein, 2002; Gunning, Halligan, & Murray, 2013; Krpan, Coombs, Zinga, Steiner, & Fleming, 2005; Maestripieri, 1999; Mills-Koonce et al., 2007). Although infants are active communicative partners, their ability to provide themselves environmental input is limited due to the fact that they are motorically immature for the first months after birth. Mothers are therefore their primary environment (Hrdy, 2000). As a consequence, experiences conveyed by the mother represent a significant part of infants' experience and thus maternal ability to interact with infants has a direct impact on the experience-developing process in infants. Thus, maternal interactive style seems crucial to consider when examining early mother-infant interactions.

Maternal interactive style refers to a set of maternal behaviors towards infants during mother-infant interactions. Maternal interactive style generally varies on a continuum between optimal maternal interactive style, suboptimal interactive style and inadequate style with respect to infants' needs. Although there is no universally accepted definition of optimal maternal interactive style, various elements of optimal maternal interactive style have been proposed.

Ainsworth and her colleagues (Ainsworth, Bell, & Stayton, 1974), for example, identified the degree of sensitivity mothers show to infant signals as a key parameter for differentiating between optimal and inadequate interactive styles. They found that sensitivity is directly related to maternal ability to perceive and interpret infant communication and to respond to infant signals appropriately and promptly. While more sensitive mothers can

perceive even subtle cues, less sensitive mothers need fairly obvious infant signals to notice them. Maternal ability to accurately interpret infant signals is based on their understanding of infant emotions, their empathy, and also their ability to see things from the infant's point of view. Even if mothers are skilled in perceiving and interpreting infant signals, the actual response (behavior) is the important component of mother-infant communication. Mothers are said to respond appropriately when they try to accommodate the infant's needs (e.g., communicating when infants try to initiate interaction, feeding infants when they are hungry, no overstimulation when infants are tired). Moreover, how fast the mother responded was identified as another important variable. This variable called "promptness" tries to reflect how shortly after the infant's signal maternal response follows.

2.1. Approaches to Study Maternal Interactive Style

As mentioned above, there are different approaches to studying mother-infant interaction. The present study investigates the relationship between Maternal Affect Attunement and oxytocin, but to date we have only data describing the relationship between oxytocin functioning and mother-infant interaction from the approach using the concept of synchrony (Feldman, Gordon, & Zagoory-Sharon, 2010; Feldman et al., 2011). Thus, I will now describe both of them, synchrony as well as Affect Attunement.

The concept of synchrony considers time as a central parameter for studying mother-infant interactions (Feldman, 2006, 2007a, 2007b). This approach involves a measurement of infants' and mothers' discrete behaviors such as facial expressions, gaze, vocalizations and touch. Synchrony in relationship between the mother's and the infant's behavior, covers three types of temporal relationships: one which is concurrent, one which is sequential and one which is organized in an ongoing patterned format (Feldman, 2007b). First, synchrony means a 'co-occurrence' or 'match' between the infant's and the mother's behaviors (Feldman, 2007b). This matching of behavior could be through the same modality, for example

mutual gazing between mother and infant, or across modalities – the matching of the mother's vocalization and the infant's smile. Researchers using this approach use conditional probabilities to examine the likelihood that one partner's (infant's or mother's) discrete behavior (e.g., a gaze) predicts the onset of the second partner's behavior (Feldman, Gordon, & Zagoory-Sharon, 2010; Feldman et al., 2011; Feldman, 2006, 2007b). Second, synchrony can be defined as a sequential relationship. The term "sequential relationship" describes the case when the behavior of one partner is followed by a behavior of the other (Feldman, 2007b). For example, positive expression from the mother such as a smile tends to elicit positive expression from the infant (Cohn & Tronick, 1987). Lag-sequential analyses are employed to assess sequential relationships (Feldman, 2007b). Third, synchrony can be described as a "dance" between mother and infant who move together in an ongoing patterned format (Feldman, 2007b). An example would be the infant's shift from disengagement to a gaze toward the mother, to which the mother would respond with a shift from quiet attention to stimulation of the infant (Feldman, 2007b). These ongoing lagged associations between the mother's and infant's stream of behaviors are measured by time-series analysis (Feldman, 2007b).

Although this approach offers significant insight into the workings of mother-infant interactions, it naturally has limitations. It can describe in detail the temporal patterns in mother-infant interactions, its rhythms and cycles, but it does not tell us much about their content. If we want to know more about what is behind the temporal pattern, behind the overt behaviors, and more about the qualitative aspects of mother-infant interactions, then we need another approach.

An alternative approach to studying mother-infant interactions focuses on the importance of sharing emotions (Legerstee et al., 2007; Markova & Legerstee, 2006; Stern, 1984; Trevarthen & Aitken, 2001). There is a variety of definitions of the terms "affect" and "emotion" in emotion literature, and a clear distinction between these concepts in emotion research is still missing (Damasio, 2000; Panksepp, 2004). Another thing to

consider is that these terms are closely linked together and in emotional experience they may interact in such a complex way that a precise description of the process is not yet possible. For these reasons, the present study will use the terms “emotion” and “affect” interchangeably (as for example Davidson, 2003) and as a superordinate category. This broad category includes (but is not restricted to) emotions as defined by Damasio: “emotions are specific and consistent collections of physiological responses triggered by certain brain systems when the organism represents certain objects or situations (e.g., a change in its own tissues such as that which produces pain, or an external entity such as person seen or heard; or the representation of a person, object, or situation conjured up from memory into the thought process.)” (2000, p. 15).

Sharing emotions with other individuals is deeply grounded in human nature (Keysers, 2011). Emotions communicate information to other people, provide us with information and get us ready to react. Emotions convey information not only about an expresser’s affective state, but also about his or her interpersonal intent (Darwin, 1998) and serve a social predictive function (Ekman, Friesen, & Ellsworth, 1972). Emotions are especially vital in the context of early mother-infant communication, because they help preverbal infants to communicate their needs, intentions, and desires (Sroufe, 1997). For instance, crying infants signal through several modalities (facial expression, body movement, and vocalization) to their mothers that they are tired or hungry. In this example, complex emotions communicate information about infant needs to mothers. When non-depressed mothers hear crying infants, their own level of arousal changes and they are motivated to soothe the infant. Their own emotions provide them with information about the situation and motivate them to act (van Kleef, 2009). Communication for healthy mothers and infants does not require extra effort; both mothers and infants express, share and respond to emotions automatically and largely intuitively (Keysers, 2011).

It has been shown that this sharing of emotions during mother-infant interactions is facilitated by maternal attunement to the infant's signals

(Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). Maternal Affect Attunement is a collection of behaviors that express the quality of feeling of a shared affect state without imitating the exact overt behavior of the inner state (Stern, 1985). In other words, mothers "get inside" of an infant's subjective experience and they let infants know that they understand it, without using words (Stern, 1985). The purpose of attunement is not only to transmit information, but also to maintain a feeling of connectedness (Stern, 1985). Highly attuned mothers express sharing of an infant's perspective, maintain their focus of attention, show positive affect, care about their needs, show respect for their interests, recognize many of their verbal and nonverbal signals, and successfully modulate their negative affect (Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). However, they may not always rank high on "similarity of response" and the level of synchrony or match between maternal and infants' behaviors is not necessarily high, because during attunement mothers focus more on internal affect state than on the overt physical behavior of infants (Stern, 1985). It should be noted that attunement occurs mainly out of awareness and almost automatically (Stern, 1985).

Regardless of the theoretical approach, several studies have shown that the coordination of mother-infant responses is incredibly rapid. Various studies found that mothers generally respond to infant behavior within a time span of 1 to 2 seconds (Keller, Lohaus, Völker, Cappenberg, & Chasiotis, 1999; Nicely, Tamis-LeMonda, & Bornstein, 1999; Papoušek & Papoušek, 1995), and some responses occur as quickly as within 200–400 ms, so that they are considered to be below the threshold of conscious awareness (Papoušek, 2000). This suggests that there may be some relatively automatic parenting responses to infant-specific sensory and behavioral cues ('intuitive parenting' responses; Papoušek & Papoušek, 1995), and that a bond of unconscious communication between mother and infant exists (Schore, 2001b). The observation that mothers respond to infant behavioural cues (such as different hand positions during various states of alertness) even

though they report being unaware of such signals also supports relative automatic patterns in mother-infant interactions (Papoušek & Papoušek, 1995).

The fact that maternal response is remarkably quick and often out of consciousness suggests the existence of neurobiological processes facilitating these maternal behaviors. Although precise descriptions of these neurobiological processes promoting maternal reaction remain unclear, it is worth noting that during pregnancy mothers undergo fundamental hormonal changes, which prepare not only their bodies for birth but also prepare the brain to focus on the care and well-being of her infant (Panksepp, 2004). Oxytocin, together with opioids and the prolactin system appear to be the key participants in this important preparation process (Panksepp, 2004).

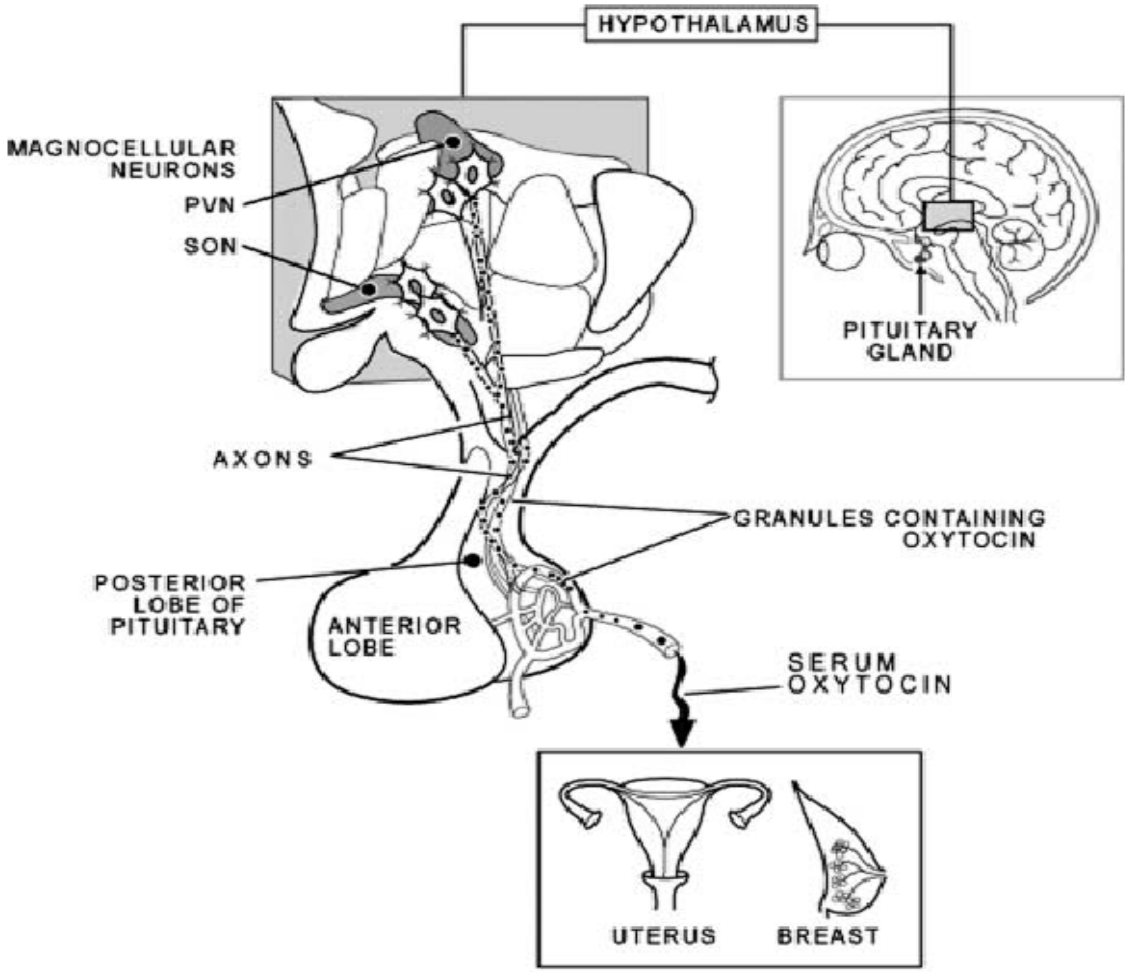
3. Oxytocin

Oxytocin is a nine-amino acid neuropeptide and its structure is very similar to arginine vasopressin, which differs in only two of the nine amino acids (Gimpl & Fahrenholz, 2001). Oxytocin and arginine vasopressin are part of an old peptide family (the oxytocin/vasopressin superfamily) that can be traced through invertebrates (Insel, 2010). These peptides affect social and reproductive behaviors in different species and they are highly conserved across species (Donaldson & Young, 2008). Nearly all vertebrates synthesize an oxytocin-like and vasopressin–like peptide (Acher, Chauvet, & Chauvet, 1995). The conservation of the reproductive and social functions of these peptides is visible across species, but the specific behaviors influenced by the peptide are species-specific (Donaldson & Young, 2008). Oxytocin and arginine vasopressin are related neuropeptide systems which not only interact together, but are also part of other neurochemicals (e.g., opiates, cortisol, estrogen) that coordinate social behaviors and stress response (Carter, Grippo, Pournajafi-Nazarloo, Ruscio, & Porges, 2008).

The present thesis will focus solely on the oxytocin system, the functional unit that contains both oxytocin and its receptor (MacDonald & MacDonald, 2010). The word oxytocin was coined from the Greek words (ω k ν ξ, τ o k ox ξ) meaning "rapid birth" (Gimpl & Fahrenholz, 2001). The chemical formula of the compound was not known until the 1950s, when the specific sequence and structure of oxytocin was first described by du Vigneaud and his colleagues (du Vigneaud, Ressler, Swan, Roberts, & Katsoyannis, 1954; du Vigneaud, Ressler, & Trippett, 1953), which enabled them to chemically synthesize the very first peptide hormone in a biologically active form (du Vigneaud et al., 1953). The classic view of oxytocin as a hormone only acting on the peripheral nervous system (important for stimulation of uterine smooth muscle contraction during labor and milk ejection during lactation) has been revised (Gimpl & Fahrenholz, 2001).

According to present knowledge, oxytocin plays dual roles: (a) as a neurotransmitter and neuromodulator in the central nervous system and (b) as a hormone on periphery (Gimpl & Fahrenholz, 2001; Insel, 1997, 2010). Oxytocin is primarily synthesized in neurosecretory cells of the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus (Gimpl & Fahrenholz, 2001; Ludwig & Leng, 2006) (see Figure 2 for details). Part of the oxytocin synthesized in the PVN and SON is transported via axonal connections to the posterior pituitary gland, from where it enters the peripheral bloodstream for its hormonal actions (Gimpl & Fahrenholz, 2001; Ludwig & Leng, 2006). Oxytocin, released to the bloodstream from the posterior pituitary gland, does not readily re-enter the brain because of the blood-brain barrier (Meisenberg & Simmons, 1983). However, possible transport mechanisms that enable oxytocin to cross the blood-brain barrier are likely to exist (McEwen, 2004).

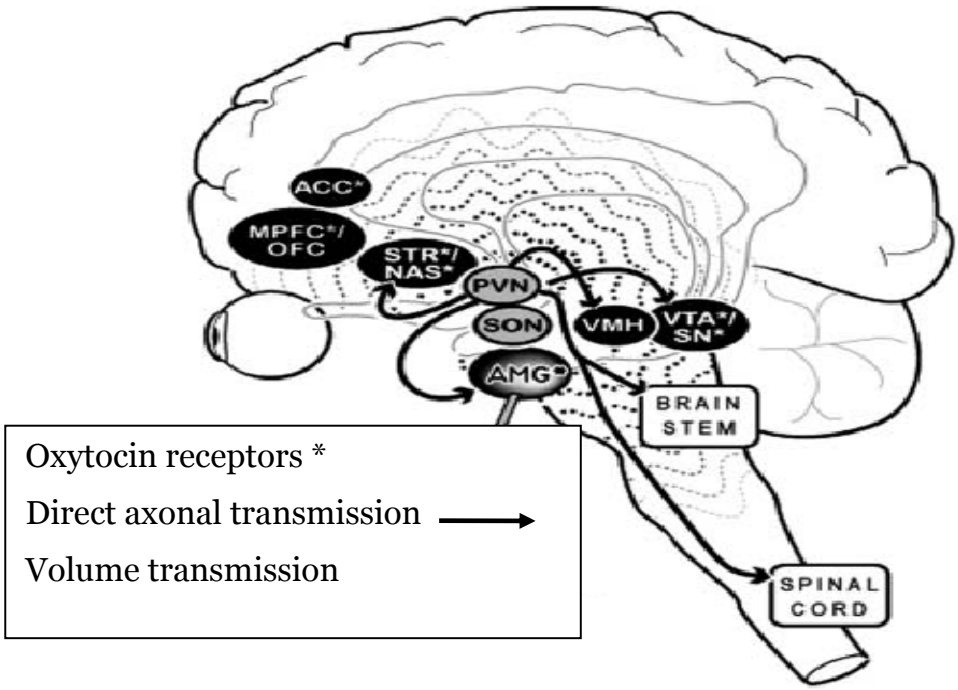
Figure 2. Oxytocin's Synthesis and its Release to the Bloodstream (MacDonald & MacDonald, 2010).



Oxytocin is primarily synthesized in magnocellular neurons of the paraventricular nuclei (PVN) and supraoptic nuclei (SON) of the hypothalamus (Ludwig & Leng, 2006). Each magnocellular neuron has one axon that projects to the posterior lobe of pituitary. In response to a broad variety of physiological stimuli including nursing, social stimuli, sexual simulation (Insel, 2010), action potentials trigger the transport of oxytocin in secretory vesicles via axonal connections from magnocellular neurons to the posterior pituitary gland (Ludwig & Leng, 2006). Each axon gives rise to about 10 000 neurosecrectory endings that are packed with vesicles containing oxytocin (Ludwig & Leng, 2006). The posterior pituitary stores and releases oxytocin to the peripheral blood stream for its hormonal actions, such as uterine contractions and milk ejection (Ludwig & Leng, 2006).

Another oxytocin pathway includes a direct axonal connection from the PVN to the critical brain areas with oxytocin receptors (see Figure 3) (Sofroniew, 1983). Apart from the direct axonal connection, specialized neurosecrectory cells (magnocellular neurons) can also release oxytocin from its soma and dendrites (Ludwig & Leng, 2006).

Figure 3. Some of Oxytocin's Pathways in the Central Nervous System (MacDonald & MacDonald, 2010).



Oxytocin is also produced by parvocellular neurons of the paraventricular nucleus, whose axonal connections remain within the brain area (Gimpl & Fahrenholz, 2001; Ludwig & Leng, 2006). Axonal connection include sites such as anterior cingulate cortex (ACC), amygdala (AMG), medial prefrontal cortex (MPFC), nucleus accumbens (NAS), orbitofrontal cortex (OFC), substantia nigra (SN), striatum (STR), ventromedial hypothalamus (VMH), ventral tegmental area (VTA), as well as the brain stem and spinal cord (MacDonald & MacDonald, 2010; Sofroniew, 1983)

This creates a "volume transmission effect" (see Ludwig & Leng, 2006, for review) where the signal is diffused in a three-dimensional space within the brain extracellular and cerebrospinal fluid and as a result, the effects of oxytocin are less directed (Agnati, Zoli, Strömberg, & Fuxe, 1995; Ludwig &

Leng, 2006). Although the exact mechanism of this somatodendritic release remains unknown, this kind of release produces huge variability in interneuronal signaling and thus oxytocin can affect very distant brain areas (Landgraf & Neumann, 2004). As with other neuropeptides, no active re-uptake mechanism for oxytocin has yet been described. It is therefore plausible that after oxytocin exerts its action on the receptors, it is deactivated through slow degradation. Oxytocin, like other peptides, can thus act over a longer period of time and over a wider area (Saeb-Parsy, 1999).

Thus far we know that oxytocin acts both on the peripheral nervous system and within the central nervous system (Gimpl & Fahrenholz, 2001; Ludwig & Leng, 2006). The peripheral pathway includes a direct axonal connection from the hypothalamus nuclei (PVN and SON) to the posterior pituitary gland, from where it enters the peripheral bloodstream. Basically two main pathways exist in the central nervous system via which oxytocin exerts its action, namely a direct axonal connection to critical brain areas and a somatodendritic release of oxytocin, which creates the "volume transmission effect".

It is worth noting that the peripheral and central effects of oxytocin are influenced not only by diversity in its production and release, but also by variations in the density and location of oxytocin receptors (MacDonald & MacDonald, 2010). To date, oxytocin has only one known receptor (Kimura, Tanizawa, Mori, Brownstein, & Okayama, 1992). Oxytocin receptor shows low ligand selectivity. This means it has only a 10-times higher selectivity for oxytocin over vasopressin, which makes arginine vasopressin a partial agonist which binds to oxytocin receptor (Kimura et al., 1997). Oxytocin receptors are distributed in the brain areas and periphery in highly species–specific fashion (Insel, 2010). In humans, receptors have been found in certain brain regions including the olfactory system, cortical areas, the basal ganglia, the limbic system, the thalamus and hypothalamus, the brain stem and peripheral systems including female and male reproductive organs, mammary glands and the thymus. The complete oxytocin receptors map is

still nonetheless waiting to be elucidated (Gimpl & Fahrenholz, 2001). Although the effects of oxytocin are strongly dependent on receptor expression, up to date research has not yet mapped individual variations of receptors, gender differences or developmental changes of oxytocin receptors in the human brain (Insel, 2010). The expression of oxytocin receptors changes during development and it is influenced by the rearing environment (Gimpl & Fahrenholz, 2001; Tribollet, Dubois-Dauphin, Dreifuss, Barberis, & Jard, 1992).

Animal studies demonstrated that early experience, including prenatal stress and postnatal social interactions, can have long-lasting effects on the oxytocin system (Carter, Boone, Pournajafi-Nazarloo, & Bales, 2009) and consequently on individual behavioral outcome. Thus, the effect of early experiences (outcomes) may possibly be mediated at least in part by the quality of mother-infant interactions, which can be associated with plasticity in oxytocin receptors as well as the quantity of synthesized oxytocin (Theodosis, 2002).

3.1. Measurement of Oxytocin

A large body of animal research measures centrally produced oxytocin in cerebrospinal fluid and oxytocin on the peripheral nervous system in plasma from blood samples (Dogterom, Van Wimersma Greidanus, & Swaab, 1977; Kendrick, Keverne, Baldwin, & Sharman, 1986; Kendrick, Keverne, Chapman, & Baldwin, 1988; Maestripieri et al., 2009; Reppert, Schwartz, Artman, & Fisher, 1983; Winslow, Noble, Lyons, Sterk, & Insel, 2002). However, there are several issues regarding measurement of oxytocin in humans.

First, to date, there is no non-invasive technology for direct measurement of oxytocin in the central nervous system. Thus, human research has to rely on peripheral measures of oxytocin. Relying on peripheral measures of oxytocin raises the important question concerning the coordination of oxytocin release in the central nervous system and the

peripheral nervous system. The behavioral effects of oxytocin are thought to reflect its activity in the central nervous system, thus when we rely on peripheral measures of oxytocin, we have to assume that levels of oxytocin on the peripheral nervous system reflect the coordination of release in the brain. Correlational studies have shown that plasma levels of oxytocin have no relationship to oxytocin levels in the cerebrospinal fluid (Günther & Landgraf, 1983; Perlow et al., 1982). Moreover, a circadian rhythm of oxytocin in humans has only been observed by measuring oxytocin in the cerebrospinal fluid, but not in plasma (Amico, Tenicela, Johnston, & Robinson, 1983). Even so, these findings do not rule out the possible coordination of central and peripheral release. It may be the case, as Ross and his colleagues (2009) hypothesize, that coordination could occur under specific situations or physiological states, such as during labor or sensory stimulation during breast feeding. They suggest that oxytocin fibers found in the forebrain may be collaterals of the magnocellular neurons projecting to the posterior pituitary. This would provide a direct mechanism for the coordination of central and peripheral release. Results from animal studies suggested that various conditions such as mating, labor, lactation or sexual activity trigger coordinated release in the central nervous system as well as the peripheral nervous system (Kendrick et al., 1988; Keverne & Kendrick, 1992; Ross et al., 2009). The complexity of the oxytocin release system possibly enables the coordination of central and peripheral release and at the same time allows independent central release (Landgraf & Neumann, 2004).

Second, few studies used blood sampling for measuring oxytocin. This method is not only costly and time consuming (Seltzer & Ziegler, 2007), but due to the interaction between oxytocin and the stress response system (Carter, 1998), the stressful situation of collecting samples can significantly influence the results.

Using less-invasive measures would thus seem essential for studying levels of oxytocin under natural conditions. Several studies use urinary samples to detect oxytocin (Bick & Dozier, 2010; Feldman et al., 2011; Fries, Ziegler, Kurian, Jacoris, & Pollak, 2005). A disadvantage of this method of

measurement is the inability to capture rapid changes in oxytocin levels (White-Traut, Powlesland, Gelhar, Chatterton, & Morris, 1998). Moreover, the study participants have to be capable of providing accurate samples, which excludes infants, fragile individuals, and certain pathological populations (White-Traut et al., 2009), making this method not suitable for studying mother-infant interactions.

Thus, for studying levels of oxytocin during the on-line mother-infant interactions and collecting samples from both mothers and infants, another method of sample collection was necessary.

Since Carter and colleagues (2007) demonstrated that oxytocin measured in saliva is a reliable biomarker of peripheral oxytocin, several studies using this collection method showed its usefulness (Feldman et al., 2011; Grewen, Davenport, & Light, 2010; van IJzendoorn, Bhandari, van der Veen, Grewen, & Bakermans-Kranenburg, 2012; White-Traut et al., 2009). Saliva sample collection is quick and minimally invasive; more samples can therefore be collected in a short period of time in order to capture changes in oxytocin levels. However, despite the developing research using saliva samples and promising results, there is still no standardized protocol for collecting saliva for measuring changes in oxytocin levels.

4. The Role of Oxytocin in Mother-Infant Interaction

In this section, a broader review of oxytocin's role in animal mother-infants interactions is followed with findings documenting the role of oxytocin in human mother-infant interactions.

What we know about the role of oxytocin in mother-infant interaction is largely based upon empirical studies from animal research. Studies in rats, macaque rhesus monkeys, sheep, prairie voles, and pigs demonstrated a consistent link between oxytocin and maternal interaction with infants (Carter, 1998; Keverne & Kendrick, 1992; Lim & Young, 2006; Maestripieri et al., 2009; Maestripieri, 1999; Ross et al., 2009). For example, several studies explored the connection between the onset of maternal behavior in rats and oxytocin (Drago, Pedersen, Caldwell, & Prange, 1986; Insel, 1992; Pedersen, Ascher, Monroe, & Prange, 1982). Before rat females become mothers, they avoid rat infants or even attack them. However, rat mothers exhibit maternal behaviors after the birth of their young such as infant retrieval, infant licking, nest building and infant nursing (Noirot, 1972). Several studies demonstrated that injection of oxytocin into the central nervous system activates the onset of rat maternal behavior in virgin female rats (Fahrbach, Morrell, & Pfaff, 1984, 1985; Pedersen & Prange, 1979). Similarly, studies with nonhuman primates showed that oxytocin may have an impact on maternal motivation to interact with their infants and the quality of maternal behavior (Saltzman & Maestripieri, 2011). For example, variation in the time that rhesus macaque mothers spent nursing and grooming their infants was associated with their plasma oxytocin levels (Maestripieri et al., 2009). Based on this strong evidence from animal experiments, the role of oxytocin in human maternal behavior and mother-infant interactions was hypothesized (Carter, 1998).

Human research exploring the connection between oxytocin and mother-infant behavior in humans faces two main obstacles. First, as it was mentioned earlier, methodological issues concerning techniques of sampling

as well as measurement of oxytocin and currently available technology for measuring oxytocin limit the research on the role of oxytocin in human behavior in general. Second, mother-infant interactions together with maternal behavior are much more complex in humans than in other species. Due to these obstacles, there is much less research on this topic in humans.

Human oxytocin research can be divided into two basic branches of studies: observational studies and studies that experimentally manipulate levels of oxytocin. Observational studies investigate the correlates of endogenous oxytocin concentration in natural settings. Researchers in these types of studies measure the levels of oxytocin in plasma, saliva or urine, as mentioned earlier. Another way to study oxytocin in humans is to administer it and directly observe its effects. These studies use a randomized, double-blind, placebo-controlled methodology. Levels of oxytocin are manipulated via intranasal administration of synthetic oxytocin. A full discussion of this methodology lies beyond the scope of this study (for exact methodology see Guastella, Mitchell, & Dadds, 2008). Although both type of studies bring useful findings, as noted by Carter (as cited in DeAngelis, 2008), experimental paradigms do not necessarily mimic natural oxytocin functioning. Moreover, because of the known effects of oxytocin in uterine contraction and breastfeeding and the unknown effect of oxytocin on the central nervous system, all studies with mother-infant dyads employed observational design (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010; Feldman et al., 2011; Feldman, Gordon, & Zagoory-Sharon, 2010; Galbally et al., 2011; Gordon et al., 2010; Levine, Zagoory-Sharon, Feldman, & Weller, 2007).

Human observational studies exploring the role of oxytocin in maternal behavior and mother-infant interactions measured mainly maternal plasma oxytocin from blood samples (Feldman et al., 2007; Levine et al., 2007; Strathearn, Fonagy, Amico, & Montague, 2009). Specifically, two recent studies examined plasma levels of oxytocin during pregnancy and maternal behavior and attachment. Both studies examined the same sample of pregnant women in three different time points - in the first and third

trimesters of pregnancy and in the first postpartum month. In the first study, plasma oxytocin levels were assayed across all time points and the mothers completed the Maternal-Fetal Attachment Scale (MFAS) in the third trimester (Levine et al., 2007). Researchers identified several patterns of change in oxytocin levels from early pregnancy to postpartum. Some mothers had stable levels of oxytocin across all time points; others showed increasing or decreasing trends. Mothers who had increasing oxytocin patterns during pregnancy tended to show higher MFAS scores. The second study examined the same sample across the same time points along with analysis of maternal behaviors and maternal representations in the first postpartum month (Feldman et al., 2007). To study maternal cognitive representations, mothers were assessed with the adapted Yale Inventory of Parent Thought and Action (Feldman, Weller, Leckman, Kvint, & Eidelman, 1999). Three composite scores were calculated: maternal preoccupation (e.g. preoccupation with infant safety), attachment representations (e.g. imagining the infant when not with her) and checking behavior (checking the infant during the day and night). To assess maternal behaviors, 15 minutes of mother-infant interaction was videotaped and coded. The authors found that plasma oxytocin levels significantly correlated with checking behavior and attachment representations. Plasma oxytocin levels also predicted the amount of maternal behaviors; the composite measure consisted of the mother's gaze at the infant's face, 'motherese' vocalizations, positive affect and affectionate touch. Findings from these two studies provide initial evidence that maternal oxytocin is associated with both cognitive and behavioral aspects of mother-infant relationships. Moreover, they point to the similar role of oxytocin in humans and other animals.

In another study, Strathearn and colleagues (2009) investigated the links between maternal own attachment style, the activation of brain reward regions in mothers and the level of oxytocin before and after interaction with their own infants. They conducted a modified version of the Adult Attachment Interview with pregnant women and divided them according to their attachment style. At 7 months after birth, they measured maternal

plasma oxytocin before and after 5 minutes of mother-infant interaction. At 11 months after birth, mothers were asked to view their own infant's smiling and crying faces during functional MRI scanning. Results showed that mothers with a secure attachment style had more elevated serum oxytocin levels after the mother-infant interaction compared to those with an insecure-avoidant attachment style. This result was strongly correlated with the activation of brain reward regions, including the ventral striatum, and the oxytocin-associated hypothalamus and pituitary region in response to their infant's emotion faces. The authors suggested that one possible explanation could be that mothers in the secure attachment group may produce more oxytocin in response to the visual cues of their infant. This, in turn, increases the experience of reward which may, in turn, motivate the mother to interact with her infant. This study sheds light on the associations between the activation of brain reward regions in mothers and levels of maternal oxytocin pre- and post- interaction with infants. With the introduction of the hypothesis about experiences of reward supporting maternal motivation to engage in interactions with infants, this study suggested another way how oxytocin could be helpful in mother-infant interactions.

Together, these findings are consistent with the hypothesis that oxytocin influences maternal behavior in humans. Although these studies provide the first insight into the relationship between oxytocin and maternal behavior, early mother-infant interactions are a mutual and bidirectional process. It has been suggested that during attuned interactions, mothers and infants coordinate not only behaviors, but also physiological rhythms (Field, 1985). Carter and colleagues (2007) demonstrated that oxytocin measured in saliva is a reliable biomarker of peripheral oxytocin. Thus, in recent studies both maternal and infants' oxytocin levels are explored in the context of mother-infant interactions by sampling saliva.

In one such study Feldman and colleagues (Feldman, Gordon, & Zagoory-Sharon, 2010) examined saliva oxytocin levels in parents and their infants at two time points: following a 10-minute familiarization period

when no touch between parent-infant interaction took place and 15 minutes after 15-minute parent-infant interaction. In addition, following a 10-minute familiarization period, parents provided plasma samples together with saliva samples. To assess parental and infants' behavior as well as affect synchrony, a parent-infant interaction was videotaped and coded. Results showed that oxytocin measured in plasma and saliva was interrelated. Infant and parental oxytocin levels were correlated at both collection points, indicating that oxytocin systems of parents and infants are interdependent both before and after interaction. Moreover, the degree of affect synchrony between parents and infants moderated the relation between parental and infant levels of oxytocin. Infant levels of oxytocin were independently predicted by parental oxytocin, but under conditions of high levels of affect synchrony infants whose parents had high oxytocin levels had a significantly higher oxytocin level than infants whose parents had relatively low oxytocin levels. However, no differences were observed in infant levels of oxytocin among infants whose parents had a high or low level of oxytocin under conditions of low affect. These findings provide initial evidence that mother and infant oxytocin systems are interrelated, oxytocin is released during the mother-infant interaction and that functioning of the oxytocin system is transmitted from mothers to infants through positive parent-infant interaction measured by affect synchrony.

Compared to other non-primate animals, human mothers have typically one infant at a time and thus maternal investment is much larger. Maternal care includes not only nutrition and protection, but also transfer of social skills that are necessary for infant survival and success in life (Saltzman & Maestripieri, 2011). The question is how exactly mothers transfer social skills to their infants. As described earlier, sharing emotions is suggested to be the main factor in mother-infant communication (Legerstee, 2005; Sroufe, 1997). Moreover, it has been shown that sharing of emotions in mother-infant interactions is facilitated by maternal attunement to the infant's signals (Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). Several studies demonstrated that Maternal Affect

Attunement may influence infant interaction skills (Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). For example, highly attuned mothers facilitate their infant's ability to progress from dyadic interaction to triadic interaction (Legerstee et al., 2007). Similarly, infants of high attuned mothers demonstrated the ability to differentiate between different interactive conditions. Compared to infants of less attuned mothers, they gazed, smiled and vocalized positively more during the natural interactions with their mothers than during the imitative and yoked interactive conditions (Markova & Legerstee, 2006).

5. Present Study

The present study combined psychological and biochemical methods to provide more insight into the role of oxytocin in mother-infant interactions. The first aim of the present study was to investigate the relationship between Maternal Affect Attunement and levels of oxytocin in mothers and infants during mother-infant communication. The second aim was to replicate previous findings concerning the interdependency of maternal and infants' oxytocin functioning (Feldman, Gordon, & Zagoory-Sharon, 2010). The third aim of the present study was to extend findings concerning the timing of sampling used in the research using salivary samples to measure levels of oxytocin in mother-infant interaction.

Mothers and their infants were observed during three conditions, namely Baseline, Natural Interaction and Modified Interactions. To assess individual maternal interactive style, Maternal Affect Attunement consisting of two dimensions, Maintaining Attention and Warm Sensitivity, was coded. To determine levels of oxytocin, saliva samples were taken both from mothers and infants at four collection points, namely pre Baseline, post Baseline, post Natural Interaction and post Modified Interaction.

For the present study two main research questions were formulated:

1. Is there a relationship between mother's levels of oxytocin and her Affect Attunement?

1. a Is there a relationship between mother's levels of oxytocin and her Maintaining Attention?

1. b Is there a relationship between mother's levels of oxytocin and her Warm Sensitivity?

2. Are infant and maternal levels of oxytocin interdependent?

The following hypothesis statements were developed from the research questions:

H_{o1A}: There is no relationship between Maintaining Attention and maternal oxytocin.
H_{A1A}: There is a relationship between Maintaining Attention and maternal oxytocin.

H_{o1B}: There is no relationship between Warm Sensitivity and maternal oxytocin.
H_{A1B}: There is a relationship between Warm Sensitivity and maternal oxytocin.

H_{o2}: There is no relationship between infants' oxytocin levels and maternal oxytocin levels.
H_{A2}: Infants' oxytocin levels positively correlate with maternal oxytocin levels.

6. Method

6.1. Participants

Overall, 64 mothers and their infants were recruited in prenatal childbirth classes and in mother-infant activity classes. Of this sample, 21 mother-infant dyads were excluded because they could not complete the experimental procedure (e.g., infants fussy, unusable saliva samples). Consequently, 43 mothers and their infants participated in the study (24 girls). Their visit to the infancy laboratory was arranged when the infants were 4 months old (*M*=137.51 days, *SD*= 17 days). All of the infants were healthy (5 minutes Apgar ratings 6-10) and born at term, with a gestation period of at least 36 weeks. The majority of infants (90.7%) had no siblings.

Mothers were 31.14 years old at infants birth (*SD*=3.85 years) had on average 5.09 years of higher education (*SD*= 2.61 years). The majority of mothers (86%) breastfed their infants. All dyads were of European Caucasian origin and came from middle to upper class families based on parental education. Mothers and infants received a small gift for participating.

6.2. Material and Procedure

In line with previous studies measuring oxytocin in mothers (Feldman et al., 2011; Feldman, Gordon, & Zagoory-Sharon, 2010), visits at the infancy laboratory were scheduled between 1 and 4pm. Mothers were asked to come at least 30 minutes after breastfeeding, because previous research showed that salivary oxytocin in breastfeeding mothers was highest 30 minutes before feeding, declined to the lowest level at the initiation of feeding and the level of oxytocin increased again 30 minutes after the end of feeding (White-

Traut et al., 2009). In addition, mothers were asked to refrain from eating or drinking (other than water) at least one hour before testing.

Initially, mothers were informed about the experimental procedure and saliva extraction, upon which they signed an informed consent. Mothers were then instructed to rinse their mouth with water to remove food residue and a first salivary sample was collected from mothers and infants. Infants were seated in an infant-seat lying on a table (95 x 65 x 50 cm). Mothers sat facing the infant (approximate eye level between mothers and infants were 30 cm), so they could engage in a face-to-face interaction. Interactions were filmed using two digital cameras. One camera focused on the mother's face and the other one on the infant's face. The two recordings were combined using the split-screen option.

The experimental procedure consisted of three main parts in a fixed order: Baseline, Natural Interaction and Modified Interactions.

Baseline. The goal of the Baseline part was to set up a condition without interaction between mothers and infants. Mothers were asked to fill out various questionnaires and refrain from communication with infants. Infants watched a Baby Einstein ® DVD designed for children from three months. The duration of the Baseline part was 10 minutes.

Natural Interaction. The goal of the Natural Interaction part was to allow observation of natural interaction between mothers and infants. Mothers were instructed to interact with their infant as they usually would at home. These face-to-face interactions could include touching, singing, talking, and singing rhymes, but not playing with toys. The duration of the Natural Interaction part was 10 minutes.

Modified Interactions. The goal of the Modified Interactions part was to set up conditions where natural interaction between mothers and infants is disrupted in various ways. Mothers were asked not to respond to the infant's signals and to interact with the infants using normal adult speech instead of child-directed speech, to speak to the researcher sitting next to the infant and to imitate their facial expressions, movements and vocalization. The analysis of Modified Interaction was not part of the present study.

6.3. Measures

Three main measures were used in the present study: (a) questionnaires as control measures, (b) Maternal Affect Attunement as a measure of maternal interactive style, and (c) oxytocin levels in infants and mothers as physiological correlates of mother-infant interaction.

6.4. Questionnaires/Control Measures

Demographic questionnaire. Mothers completed a demographic questionnaire, providing information about their age, education and feeding style (breastfeeding vs. non- breastfeeding), as well as infant age and health history.

Postnatal depression. Postnatal depression has a negative impact on mother-infant interaction and maternal interactive style (Field, 2010). Thus mothers were screened for symptoms of depression with the Beck Depression Inventory (BDI-II; Beck, Steer, & Brown, 1996, 1999). The BDI-II is a 21-item self-report instrument, where each item is rated on Likert-type scale from 0 (absence of symptom) to 3 (severe or persistent presence of the symptom). A total score is obtained by summing the ratings for all items, and can range from 0 to 63. Symptom severity was coded according to the scoring guideline: scores within the range of 0 to 13 are considered as minimal or no symptoms of depression, scores 14 to 19 as mild depression, 20 to 28 as moderate depression and 29 to 63 as severe depression.

6.5. Maternal Affect Attunement

To assess individual maternal interactive style, Maternal Affect Attunement was coded. Maternal Affect Attunement is defined by two dimensions, Maintaining Attention and Warm Sensitivity (Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006), and has been

shown to reliably identify individual differences in the degree to which mothers tune in with their infants (Bartling et al., 2010).

Maintaining Attention was defined as a verbal or nonverbal response that follows and supports the infant's self-selected interest, without attempt to redirect the infant's attentional focus. It is not important if infants try to catch the mothers' attention or not. By Maintaining Attention, mothers express sharing an infant's perspective. The duration of Maintaining Attention was coded on a second-by-second basis. For example, if infants looked at the camera in the laboratory and mothers responded by looking at the camera and asked "What is it over there? Do you like the camera?" The coding of Maintaining Attention started when mothers looked at the camera and ended when mothers or infants changed the subject of attention. The proportion of time in which mothers maintained attention in Natural Interaction part was calculated as a percentage.

Warm Sensitivity comprised three composites: Positive Affect, Warm Concern and Social Responsiveness (Bartling et al., 2010; Landry et al., 1998; Legerstee et al., 2007; Markova & Legerstee, 2006). Global ratings of each category were made once every minute during the Natural Interaction part. Ratings were recorded on a five point scale ranging from a low presence of the behavior (1) to an extensive presence of the behavior (5), and an average was calculated for each composite.

Positive Affect referred to a warm tone of voice, the use of affective words and the intensity and duration of the mothers' affective behavior such as positive facial expression. Mothers rating high on Positive Affect expressed positive affect most of the time. They smiled often, used a warm tone of voice and child-directed speech with affective words.

Warm Concern was defined as the mothers' care about the infant's needs and sensitive behavior during play with a respect for the infant's interests. High warm concern was coded when mothers were in a close contact with their infants, touched them in an affectionate or loving manner and cared for their safety and comfort.

Social Responsiveness described mothers' prompt responses to the infants' signals, such as smiling and vocalization as well as modulation of any negative infant behavior. High social responsiveness was coded when mothers recognized many of the infant's verbal and nonverbal signals, imitated the infant's behavior most of the time and/or modulated the child's negative affect successfully.

Reliability. To determine inter-rater reliability on coding Maternal Affect Attunement, Cohen's kappa statistic was calculated. One rater coded all the data and the second rater coded 30 % of the randomly selected data. Interrater reliability reached following kappas: 0.842 for Maintaining Attention, 0.789 for Warm Sensitivity, 0.823 for Positive Affect, 0.786 for Warm Concern and 0.760 for Social Responsiveness.

6.6. Oxytocin Collection and Analysis

During the visit, a total of four saliva samples from each mother and infant were collected to determine the concentration of oxytocin. The first sample was collected before the Baseline part, the second sample after the Baseline part, the third sample was obtained after the Natural Interaction part, and mothers and infants provided the fourth sample after the Modified Interactions part.

Saliva samples were collected using oral swabs. The mothers were instructed to keep swabs (Salimetrics Oral Swab) under their tongue for two minutes. A research assistant collected saliva samples from the infants, holding one end of a swab specially designed for infants (Salimetrics Infant's Swab) while the infants sucked the other end. The swabs were put into the collection tube after the collection (Swab Storage Tube) and kept on ice in a thermocol ice box during the whole procedure. After the procedure collection tubes were frozen and stored at −20°C.

The present study used a similar method to measure oxytocin in saliva as the study validating measurement of oxytocin in saliva (for exact methodology see Carter et al., 2007). However, because measurement of oxytocin in saliva was a relatively new approach without any standardized protocol for collection, a pre-experiment was conducted. The pre-experiment had two main goals: The first goal was to investigate the volume of saliva recovered from swabs. The second goal was to determine the concentration range of oxytocin in the saliva samples. A commercially available kit (Oxytocin EIA kit, ADI-901-153, Enzo Life Science) was used to determine the concentration of oxytocin in 10 test samples (5 adults and 5 infants). The EIA kit is a competitive enzyme immunoassay for the quantitative determination of oxytocin. In the kit, oxytocin in samples competes with added oxytocin attached to alkaline phosphatase for polyclonal antibody to oxytocin. The assay procedure results in colorization of the sample with yellow. The intensity of the colorization is inversely proportional to the concentration of oxytocin in either standards or samples. Instructions stated that the limit for detection of the assay was 11.7pg/mL.

Saliva was recovered from the swabs by centrifugation (2500× g for 10 min at 4°C). The samples were measured directly without any further modification and the assay procedure was performed meticulously following the kit's instructions without any modification. All test samples were run in duplicates and a separate standard curve was constructed for each plate. After the first part of the assay procedure, the plate with reagents was incubated overnight at 4°C. The second part of the procedure took place the following day, after which the plate was incubated at room temperature for 1 hour. The reaction was then stopped and the optical density of the samples was immediately read on a microplate reader at 405nm. The concentrations (in pg/mL) of oxytocin were calculated from the relevant standard curve using Softmax Pro 5.2. Each standard curve was checked for quality control parameters as they were stated in the instructions. The results from the pre-experiment showed that all the test samples in the pre-experiment had sufficient volume concentrations (at least 1 mL) and were above the limit of

detection of the assay. The intra-assay coefficient of variability was 13.28%. Thus, unlike in previous research (Carter et al., 2007; Feldman et al., 2011), it was not necessary to concentrate the samples before assay. Since the pre-experiment was a success, exactly the same procedure was used in the main analysis of saliva samples.

7. Results

The findings of the study are reported in two sections. The results from data screening, the analysis of control measures, a correlational analysis of Maternal Affect Attunement and descriptive statistics are presented in the preliminary analyses. The second section presents the hypotheses of the study.

7.1. Preliminary Analyses

Prior to data analysis, all data was screened for deviation from a normal distribution as well as univariate outliers (z >± 3). If univariate outliers were present, they were assigned a new score one unit higher/lower than the next highest/lowest score in the distribution (Tabachnick & Fidell, 2001). One outlier was found in maternal warm concern. The following outliers were found in maternal levels of oxytocin: three outliers in the first saliva sample, two outliers in the second sample, three outliers in the third sample and one outlier in the fourth sample. Three outliers were found in the first sample of infants' levels of oxytocin, one outlier in the second sample, two outliers in the third sample and one outlier in the fourth sample. After the assignment of a new score, all data was normally distributed.

Table 1 presents descriptive statistics for all main study variables, including components of Affect Attunement and salivary oxytocin measured in infants and mothers.

Table 1. Descriptive Statistics for the Main Variables of the Present Study

	M	*SD*	*range*
	MATERNAL VARIABLES		
Affect Attunement			
Maintaining Attention	69.987	23.592	18.25 – 100
Positive Affect	4.392	.266	4 – 4.89
Warm Concern	4.379	.324	3.66 – 5
Social Responsiveness	4.153	.470	3.20 – 5
Oxytocin			
OXT 1	158.216	88.810	29.433 – 324
OXT 2	164.683	99.654	17.389 – 356
OXT 3	165.049	91.171	12.773 – 332
OXT 4	158.481	99.152	36.702 – 324
	INFANT VARIABLES		
Oxytocin			
OXT 1	146.015	86.277	35.130 – 284
OXT 2	190.978	112.744	29.724 – 397
OXT 3	178.169	108.761	11.498 – 375
OXT 4	158.454	73.904	52.044 – 297

Note: Values of oxytocin are in pg/mL; m=maternal oxytocin level; i= infant's oxytocin level; OXT1=pre Baseline collection point; OXT2=post Baseline collection point, OXT3=post Natural Interaction collection point, OXT4= post Modified Interaction collection point

Control measures. In order to control for possible confounds, background variables including maternal age, education, feeding style (breastfeeding vs. non-breastfeeding), infants' age and gender were examined in relation to all main variables, namely to maternal and infant's levels of oxytocin and Affect Attunement. A correlational analysis showed no significant correlations. Thus, none of the potential confounds were included in further analyses.

Postnatal depression. The majority of mothers (84%) showed minimal or no symptoms of depression. However, 5 mothers showed symptoms of mild depression, one mother showed symptoms of moderate depression and one mother showed symptoms of severe depression as measured by the BDI-II. It is important to note that the BDI-II is designed as a screening tool rather than a diagnostic tool for depression. However, because postnatal depression was thought to possibly have an impact on mother-infant interaction, a correlational analysis was performed between scores on the BDI-II and composites of Maternal Affect Attunement. The results showed no significant correlations, suggesting that scores on the BDI-II had no effect on Maternal Affect Attunement. No significant correlations between the BDI-II and maternal and infant oxytocin were found.

Maternal Affect Attunement. To evaluate the relation between the components of maternal Warm Sensitivity, a correlational analysis was performed. As can be seen in Table 2, significant or marginally significant correlations were found between the three components of Warm Sensitivity. Thus, a mean score of Warm Sensitivity was computed from Positive Affect, Warm Concern and Social Responsiveness (M = 4.303, SD = .285, *range* = 3.72 – 4.86).

The analysis also revealed that a mother's Maintaining Attention and her Warm Sensitivity were significantly correlated, $r(43) = .591$, $p < .001$. This indicates that the two factors in fact assess a similar underlying capability, namely Maternal Affect Attunement. Maintaining Attention

and Warm Sensitivity are, however, hypothesized to be qualitative distinct constructs (Bartling et al., 2010; Landry et al., 1998) and thus all following analyses were conducted with both factors separately.

Table 2. Correlations Between Components of Warm Sensitivity

	1.	2.	3.
1. Positive affect	-		
2.Warm concern	.418*	-	
3.Social responsiveness	.278†	.536**	-

Note. †p < .06. *p < .01. **p < .001.

7.2. Hypotheses-testing

Relationship between a mother's levels of oxytocin and her Affect Attunement. To test the hypothesis concerning the relationship between a mother's oxytocin levels and her Affect Attunement, an Analysis of Covariance (ANCOVA) was performed on maternal oxytocin level with time (the first, second, third and fourth collection point) as the within-subjects factor, and Maintaining Attention and Warm Sensitivity as the covariates. The results revealed a significant effect of Warm Sensitivity, $F(1, 21) = 5.979$, $p = .023$, $\eta^2 = .222$, showing that Warm Sensitivity was significantly related to maternal oxytocin levels, irrespective of time. No other effects were significant.

A correlational analysis further indicated that maternal oxytocin level at all collection points as well as overall mean oxytocin level across all collection points were negatively related to maternal Warm Sensitivity (see Table 3).

Table 3. Bivariate Correlations Between Components of Affect Attunement and Maternal Oxytocin level

	OXT 1	OXT 2	OXT 3	OXT 4	OXT Mean
Maintaining Attention	.011	-.135	-.116	-.100	-.043
Warm Sensitivity	-.346*	-.422**	-.379*	-.362*	-.436*

Note. *p < .05. **p < .01. OXT1=pre Baseline collection point; OXT2=post Baseline collection point, OXT3=post Natural Interaction collection point, OXT4= post Modified Interaction collection point

Relationship between infants' oxytocin levels and maternal oxytocin levels.

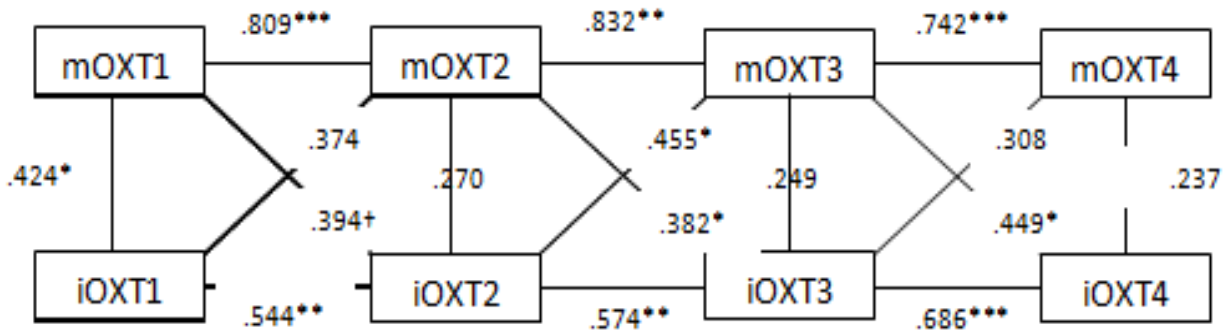
Another aim of the present study was to examine the relationship between maternal and infant oxytocin levels during various conditions, and to extend previous findings concerning the interdependency of maternal and infant oxytocin functioning (Feldman, Gordon, & Zagoory-Sharon, 2010).

Figure 4 shows the intercorrelations among the four oxytocin samples in infants and mothers. The results showed both maternal as well as infant oxytocin levels were highly correlated between the four collection points. The absolute values of the correlations among maternal oxytocin levels across measurement ranged from .742 to .832; and the infant levels of oxytocin correlations ranged from .544 to .686. Correlations were also computed between the maternal oxytocin level and the infant oxytocin level at every individual collection point. Infants' oxytocin level significantly correlated with maternal oxytocin level at the first collection point. Results also showed significant correlations between maternal and infant mean oxytocin levels across all four collection points. All other synchronized correlations were not significant.

To investigate possible direction of causality between maternal and infant oxytocin levels, cross-lagged correlations were additionally computed. As seen in Figure 4, maternal levels of oxytocin at the first, second and third previous collection point were significantly correlated

with infants' oxytocin levels at the second, third and fourth collection point, respectively. Moreover, infant's oxytocin level at the second collection point significantly correlated with maternal oxytocin at third point. All other cross-lagged correlations were not significant.

Figure 4: Intercorrelations Between Measures of Oxytocin



Note: †p < .06. *p< .05, **p < .01, ***p < .001

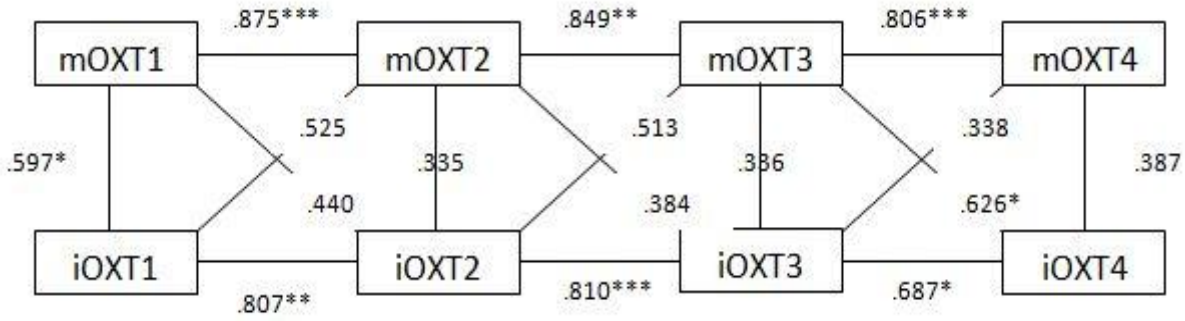
m=maternal oxytocin level; i= infants oxytocin level; OXT1=pre Baseline collection point; OXT2=post Baseline collection point, OXT3=post Natural Interaction collection point, OXT4= post Modified Interaction collection point

Because Warm Sensitivity was related to maternal oxytocin levels, intercorrelations controlled for Warm Sensitivity were also computed intercorrelations. As can be seen from Figure 5, both maternal as well as infant's oxytocin levels were highly correlated between the four collection points. The absolute values of the correlations between maternal oxytocin levels across the measurement ranged from .806 to .875; and the infants levels of oxytocin correlations ranged from .687 to .810. Infants' oxytocin level was significantly correlated with maternal oxytocin level only at the first collection point.

As can be seen from the cross-lagged correlation analysis maternal levels of oxytocin at the third collection point were significantly correlated with infants' oxytocin level at fourth collection point. All other cross-

lagged correlations between oxytocin levels at previous collection point to oxytocin level at following collection were not significant.

Figure 5. Intercorrelations Between Measures of Oxytocin Controlled for Warm Sensitivity



Note: *p< .05, **p < .01, ***p < .001

m=maternal oxytocin level; i= infants oxytocin level; OXT1=pre Baseline collection point; OXT2=post Baseline collection point, OXT3=post Natural Interaction collection point, OXT4= post Modified Interaction collection point

8. Discussion

The purpose of the present study was to provide more insight into the role of oxytocin in mother-infant interactions, extend findings concerning the timing of sampling used in the research using salivary samples to measure levels of oxytocin in mother-infant interaction and to investigate two hypotheses concerning relations between Maternal Affect Attunement and levels of oxytocin in mothers and their infants. Mothers and their infants were observed during three conditions, namely Baseline, Natural Interaction and Modified Interactions. Maternal Affect Attunement, consisting of two dimensions - Maintaining Attention and Warm Sensitivity - was coded from Natural Interaction part. Levels of oxytocin were measured from saliva samples taken both from mothers and infants at four collection points. The first three of these samples were taken in ten-minute intervals (before the Baseline condition, after the Baseline condition and after the Natural Interaction condition) and the last was taken nine minutes after the Modified Interaction condition

The research of oxytocin examines the role of oxytocin in two contexts. First, levels of oxytocin are measured as a baseline measure reflecting overall oxytocin functioning in individual. Second, oxytocin reactivity is measured as a difference between two levels of oxytocin measured before and after specific tasks or situations.

One aim of the study was to extend findings concerning the timing of sampling used in the research using salivary samples to measure reactivity of levels of oxytocin in mother-infant interactions. Very little data is available on the appropriate timing of sampling. Preliminary results of the present study indicated that that there were no significant differences between maternal or infant oxytocin levels at the four collection points. Studies examining the reactivity of oxytocin in saliva samples showed that oxytocin levels increased after massage in adults

(Carter et al., 2007) and also after warm touch intervention in adult couples (Holt-Lunstad, Birmingham, & Light, 2008). Two other studies examined the reactivity of oxytocin in the context of parent-infant interaction. In the first study, both paternal and infant oxytocin levels measured in saliva increased following 15minutes contact parent-infant interaction (Feldman, Gordon, & Zagoory-Sharon, 2010). Interestingly, the second study found an increase in salivary oxytocin following 15minutes of mother-infant interaction only in parents who provided high levels of tactile contact to their infants. No increase was found among parents who provided low tactile contact (Feldman, Gordon, Schneiderman, et al., 2010). Taken together, these findings suggest that touch may be the main component which raises the levels of oxytocin after parent-infant interactions.

Two differences in procedure between the present study and the two studies mentioned above may account for null findings concerning reactivity of oxytocin in mothers and infants. First, the length of the Natural Interaction condition in the present study was 10 minutes. Thus it is possible that in order to detect differences in levels of oxytocin with salivary measure, conditions of at least fifteen minutes may be necessary. Second, before the Natural Interaction condition mothers were instructed to interact with their infant as they usually would at home. Interactions could include tactile contact, but there were no instructions to emphasize the importance of contact. However, in the studies of Feldman and colleagues (2010; 2010) parents were asked to engage in "play-and-touch" interaction. It may be the case that different instructions could influence the level of touch which parents used in their interaction with the infant and this in turn could increase post - interaction oxytocin level. Overall, because preliminary results of the present study indicated that that there were no significant differences between maternal or infant oxytocin levels at the four collection points, it is more plausible to consider measured oxytocin levels as a reflection of overall oxytocin functioning in mothers and infants.

Previous studies indicate high levels of individual stability in measured oxytocin concentration across assessment in both adults and infants (Feldman, Gordon, & Zagoory-Sharon, 2010; Gordon et al., 2010), therefore when oxytocin is not measured during peak experience (e.g., breastfeeding), oxytocin concentration has been suggested to be a relatively stable trait of individuals (Feldman, Gordon, & Zagoory-Sharon, 2010).

Another aim of the present study was to investigate the relationship between maternal levels of oxytocin in different conditions and Maternal Affect Attunement. Surprisingly, results showed that maternal oxytocin levels at all collection points as well as overall mean oxytocin level were negatively related to maternal Warm Sensitivity. Although it is unclear why the negative relationship between Warm Sensitivity and maternal levels of oxytocin was found, there are several possible explanations for this result.

First, it is possible that both oxytocin levels and Warm Sensitivity may be related to a third underlying variable. It may be the case that this third variable is influenced by the stress reactivity. Oxytocin is released in response to stress (Carter, 1998) and acts as a neuromodulator of the stress response, however its exact role in modulating reactions of the organism to stress is still unknown (Heinrichs, Baumgartner, Kirschbaum, & Ehlert, 2003; Uvnäs-Moberg, Ahlenius, Hillegaart, & Alster, 1994). Oxytocin pathways and receptors were found in brain areas responsible for stress and anxiety and the paraventricular nucleus (also the place of oxytocin's synthesis) represents the main regulator of the HPA axis (Herman, Flak, & Jankord, 2008). One proposed way how oxytocin could be helpful in mother-infant interaction was that oxytocin reduces neophobia and feelings of stress (Carter, 1998). Perceived stress during the first months after infant birth negatively influences maternal sensitivity to their infants (Mills-Koonce et al., 2011). Therefore an increase in expression of oxytocin receptors in different brain areas as well

as an increase in the release of oxytocin in the central nervous system around the time of labor and the lactation period(Insel& Shapiro, 1992; Neumann, Russell, & Landgraf, 1993) may represent a possible way in which oxytocin with its anxiolytic effects could play a helpful role for mothers in managing stress and therfore mediating maternal sensitivity. There is evidence that oxytocin reduces the responsiveness of the HPA axis to a wide variety of physical, emotional and social stressors and this adaptive mechanism is responsible for lower levels of cortisol (used as a measure of stress) after exposure to stressors(Ditzen et al., 2009; Heinrichs et al., 2003; Heinrichs & Gaab, 2007; Neumann, 2002, 2008; Uvnäs-Moberg et al., 1994). However, there is also data showing a positive relation between oxytocin and cortisol (Taylor et al., 2006; Tops, van Peer, Korf, Wijers, & Tucker, 2007; Turner et al., 2002). Thus, Tops and colleagues (2007) suggest that the direction of the relationship between social distress and oxytocin levels found in different studies depends on context effects. The negative relationship between oxytocin and cortisol has been found after positive social interaction and positive relationship between oxytocin and cortisol have been found in context of distress. It may be the case that in context of distress cortisol-induced released of oxytocin may be seen (Tops et al., 2007). It may also be the case that these mixed results concerning the role of oxytocin in stress can just reflect the complex release patterns of the oxytocin system (MacDonald & MacDonald, 2010). To summarize, the relationship between oxytocin and stress reactivity in humans is complex and is waiting to be elucidated. However, we can speculate that a possible explanation for results found in the present study can therefore be based upon a different impact of stress each mother had during the visit in the infancy laboratory and thus the results may reflect a cortisol-induced release of oxytocin. It is possible that mothers whose behavior was coded high on Warm Sensitivity experienced less stress during the time in the infancy laboratory. On the other hand, mothers whose behavior was coded low on Warm Sensitivity experienced more stress, which should have raised their cortisol and oxytocin levels. It

is important to note, that this may be the case only if mothers experienced stress in very early in the procedure, because oxytocin from the first collection point already correlated significantly with Warm Sensitivity. Further studies employing various measures of stress (e.g., subjectively perceived stress by the mother measured by the questionnaire, salivary cortisol as a biomarker of psychological stress or measure of vagal tone in mothers and infants) are needed to verify this speculation.

The direction of the relationship between maternal oxytocin and Warm Sensitivity may at first seem to be inconsistent with Feldman's (2010) finding, which showed a positive relationship between maternal oxytocin level and mother-infant affect synchrony. However, as it was mentioned earlier, there are several differences between the construct of mother-infant affect synchrony and maternal Affect Attunement. The most important difference is that the concept of mother– infant synchrony considers time as a central parameter for studying mother-infant interaction and the construct is relational. Specifically, affect synchrony in the Feldman and colleagues (2010) study was defined as the proportion of time parent and infant coordinated their positive engagement and was expressed as a conditional probability. In contrast, Maternal Affect Attunement considers shared affect without imitating the exact overt behavior of the inner state(Stern, 1985) as a central parameter. Moreover, maternal Affect Attunement is not relational and is coded purely from mothers's behaviors. Thus, the present findings are novel and findings about the relationship between maternal oxytocin level and Maternal Affect Attunement could not be compared directly with findings measuring parent-infant synchrony.

One might also argue that based on studies that experimentally manipulated levels of oxytocin and showed that oxytocin enhances the recognition of facial expressions (for a meta-analysis, see van IJzendoorn & Bakermans-Kranenburg, 2012), a positive relationship between Warm Sensitivity and natural oxytocin in mothers should be expected. As described earlier, emotional expressions of the infants and the mothers

function to allow them to mutually regulate their interactions (Tronick, 1989). Recognizing infants' cues is a necessary condition for a proper maternal responsiveness and the ability to infer the internal state of infants enables mothers to attune their affect state to the infant's state. Several studies, using intranasal administration of synthetic oxytocin, tested the hypothesis that oxytocin might promote the ability to read another person's emotion, and showed that oxytocin enhanced the recognition of facial expressions (Averbeck, Bobin, Evans, & Shergill, 2012; Domes, Heinrichs, Michel, Berger, & Herpertz, 2007; Guastella et al., 2010, 2008; van IJzendoorn & Bakermans-Kranenburg, 2012). One possible explanation for these findings is that oxytocin enhances eye gaze. In support, Guastella et al. (2008)showed that participants treated with oxytocin had a higher number of fixations and had higher total gaze time towards the eye region. This finding may be important also in the context of mother-infant interaction, because the amount of eye gaze predicts one's ability to interpret the intentions of others (Garrett, Menon, MacKenzie, & Reiss, 2004; Klin, Jones, Schultz, Volkmar, & Cohen, 2002), and maternal faces and eyes are the most salient stimuli for infants. Taken together, these results suggest the involvement of oxytocin in emotion recognition and thus a potentially important role of oxytocin in sharing emotions may be proposed. However, all above mentioned studies using intranasal administration of synthetic oxytocin included male participants, predominantly from the clinical population (mainly diagnosed with autism, Asperger's disorder or schizophrenia). Moreover, levels of oxytocin were manipulated via intranasal oxytocin administration, thus no data is available on natural oxytocin functioning and emotion recognition. For that reason, connecting the findings from observational studies and studies that experimentally manipulate oxytocin levels needs to be done carefully. Moreover, even if mothers are skilled in perceiving and interpreting the infant's signals, the actual response (behavior) is the important component of mother-infant interaction. In coding Warm Sensitivity, only the mothers' actual behavior was coded.

A second possible explanation of finding that maternal oxytocin levels at all collection points as well as overall mean oxytocin level were negatively related to maternal Warm Sensitivity could be related to a third underlying variable representing a personal trait, which also includes behaviors coded on Warm Sensitivity. The findings concerning the negative relationship between oxytocin and Warm Sensitivity are more consistent with those of Turner and colleagues (Turner, Altemus, Enos, Cooper, & McGuinness, 1999) who found a positive relationship between plasma oxytocin in single women and unhealthy interpersonal traits like intrusiveness, anxiety and coldness. Similarly, another study reported a positive association between oxytocin measured in serum and psychoticism measured by the Eysenck Personality Questionnaire in diabetic patients. Individuals scored high on psychoticism are described as solitary, emotionally cold, impersonal, hostile, and with poor social relations (Kontoangelos et al., 2012).

These findings, together with the findings of the present study could first appear to be surprising, however they support the suggestion by Bartz and colleagues (2011) that paradoxical results across studies should not be seen as 'noise,' but as clues to the context- and person-dependent nature of oxytocin functioning.

Another finding of the present study was that there was no relationship between maternal levels of oxytocin and the second dimension of Affect Attunement - Maintaining Attention. Previous literature found mixed results concerning the relationship between Maintaining Attention and Warm Sensitivity. In several studies a positive correlation between Maintaining Attention and Warm Sensitivity was found (Legerstee & Varghese, 2001; Markova & Legerstee, 2006, Varghese, 1999).Even though that significant positive correlation between these two constructs was found in the present study, the results showed significant correlation between Warm Sensitivity, but not Maintaining Attention, and levels of oxytocin. This finding is in line with literature showing that Maintaining Attention and Warm Sensitivity are

qualitatively different constructs (Bartling et al., 2010; Landry et al., 1998). Another factor to consider is that Warm Sensitivity consisted of three composites thus representing more broad measure, while Maintaining Attention measures only one specific factor of maternal interactive style.

The second aim of the present study was to assess the relation between maternal and infant level of oxytocin across different conditions. Consistent with previous findings concerning the interdependency of maternal and infant oxytocin functioning (Feldman, Gordon, & Zagoory-Sharon, 2010), all correlations between infant and maternal oxytocin levels were positive, although some were insignificant. Most importantly, there were significant synchronized correlations between maternal and infant oxytocin level at the first collection point. This finding suggests that various interactive conditions during the procedure may interrupt the joint functioning coordination of the mother-infant oxytocin system. Procedure consisted of three various conditions (Baseline part, Natural Interaction part, Modified Interactions part) and it is possible that different tasks in conditions and shifting between various interactive conditions may disorganize mutual coordination of physiological rhythms in mothers and their infants.

To investigate a possible direction of causality between maternal and infant oxytocin levels, cross-lagged correlations were computed. The results suggest that maternal levels of oxytocin at the previous collection point may influence infants' oxytocin level at the following collection point. However, because infant oxytocin level correlated at the second collection point with maternal oxytocin at third point, this may have indicated mutual influence. Results also showed that controlling for Warm Sensitivity affected these cross-lagged correlations. Specifically, only maternal levels of oxytocin at the third collection point were significantly correlated with infants' oxytocin level at the fourth collection point. One possible explanation for this finding is that mothers with high oxytocin

had infants who were more secure after the rather distressing Modified Interaction part. However, when we bear in mind the possibility that high oxytocin levels in mothers can account for less Warm Sensitivity, a potential alternative explanation for these oxytocin findings is that mothers with more stress during the procedure may have had infants who were themselves more in stress after the rather stressful Modified Interaction part.

8.1. Limitations

A main limitation of the present study is the peripheral measure of oxytocin, specifically the measure of oxytocin in saliva. Due to the fact that the exact coordination mechanism between oxytocin release in the central nervous system and the peripheral nervous system is not fully understood, caution should be taken in interpreting the relationship between oxytocin measured on the periphery and psychological variables. However, it is important to note that from animal studies we know that under particular conditions, such as mating, labor, lactation or sexual activity there is a coordinated release of oxytocin in the central nervous system as well as the peripheral nervous system(Kendrick et al., 1988; Keverne & Kendrick, 1992; Ross et al., 2009). As stated earlier, several theoretical mechanisms of release coordination were also hypothesized (Landgraf & Neumann, 2004; Ross et al., 2009). In sum, although it is useful to explore oxytocin levels in the peripheral nervous system under different conditions (Gordon, Martin, Feldman, & Leckman, 2011), this kind of measurement gives us only a limited picture of the complex oxytocin system. Moreover, because measuring oxytocin in saliva still has no standardized protocol for collecting saliva and preparing samples for measurement, it is difficult to quantitatively compare measurements between research studies using different sampling procedures, sample preparation and type of assays. Samples in the present study were measured directly without an extraction step and without concentrating the samples. The absolute

concentration of oxytocin in the present study tended to be much higher, i.e., 11.5 to 397 pg/mL than reported oxytocin concentration in previous saliva samples (Feldman et al., 2011; Feldman, Gordon, & Zagoory-Sharon, 2010). The results found in the present study reflect more absolute concentrations measured in studies using plasma samples. However, similar differences in absolute oxytocin levels were observed in previous studies measuring oxytocin in plasma, where values with the old antibody in the commercially available EIA kit and without extraction tended to be much higher, compared to values with extracted samples and the new antibody. Thus it is plausible that measuring samples directly without an extraction step and without concentrating the samples may influence the absolute concentration. Consequently, comparing the absolute values of oxytocin concentrations is not possible. These findings indicated that absolute values of oxytocin concentration are dependent on the antibody used in the EIA kit and whether an extraction step or dry-down procedure was done. Therefore, future studies should combine methods measuring oxytocin in the peripheral nervous system with methods measuring brain activation in regions enriched with oxytocin receptors in order to get a more complex picture of oxytocin functioning and to bridge findings from different areas.

The second limitation of the present study is that the present study is correlational. Thus, it is difficult to conclude from present data whether the oxytocin levels are in fact a result or a cause of the maternal interactive style measured by the composite of Warm Sensitivity. Both oxytocin levels and maternal interactive style may be related to a third underlying variable.

The third limitation of the study is that the final sample size of 43 mother-infant dyads was quite modest. This research was conducted in an infancy laboratory and several mother-infant dyads were excluded because they could not complete the experimental procedure. Moreover, recruitment strategies resulted in over-recruitment of first-time mothers,

so findings need to be confirmed in future studies with a larger, more balanced sample.

8.2. Conclusion

Although animal research shows substantial contribution of biological factors to the quality of mother-infant interactions (Carter, 2005; Insel, 1992, 2010; Saltzman & Maestripieri, 2011), a large body of human research on mother-infant interaction has been focused on studying social and psychological factors (Galbally et al., 2011). The present study contributes to a limited amount of research studies on mother-infant interaction which also examined biological factors to provide more insight into the contribution of neuroendocrinological factors in mother-infant interaction. The present study is the first to explore the relationship between maternal Affect Attunement and levels of oxytocin in mother-infant dyads under different conditions of interaction. It also extend previous findings concerning interdependency between maternal and infant level of oxytocin (Feldman, Gordon, & Zagoory-Sharon, 2010) by showing that infant and maternal oxytocin levels were positively correlated, but significant synchronized correlations were found only at the first collection point.

Although the present study shed light on the biological mechanisms underlying mother-infant interaction, much work remains to be done. The importance of studying oxytocin in the context of mother-infant interaction is apparent from two main points.

First, we already know from animal studies that oxytocin plays a key role in “quality maternal care” (Panksepp, 2004) and recent human studies need to continue to add more specific knowledge about more complex roles of oxytocin in human mothers (Carter, 1998; Feldman et al., 2011, 2007; Feldman, Gordon, & Zagoory-Sharon, 2010; Gordon et al., 2010; Panksepp, 2004).

Second, recent studies suggest that oxytocin exhibits valuable therapeutic potential in psychiatry, e.g. in disorders such as autism, anxiety disorders, depression, and schizophrenia (for review, see MacDonald & Feifel, 2013). One of the crucial factors which influence an individual's response to oxytocin (either its own endogenous oxytocin or administered in the form of a drug) later in life, is early mother-infant relations (Bakermans-Kranenburg et al., 2012; van Ijzendoorn et al., 2011; for review, see MacDonald, 2013).

Thus, carefully studying different factors of mother-infant interaction together with levels of oxytocin may help us provide more answers to questions concerning causes of dysfunctional mother-infant interactions and strategies for changing dysfunctional aspects of sharing emotions in mother-infant dyad and reestablishing healthy mutual interactions.

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10. List of Appendices