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# Effect of oxytocin and cortisol levels on mother-infant bonding

Jana Olivova\*, Department of Psychology, Charles E. Schmidt College of Science, Florida Atlantic University, Boca Raton, FL 33431.United States.jolivova@fau.edu

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#### Abstract

Oxytocin (OT) has been associated with mother-infant bonding, while cortisol levels have been associated with stressful response. The objective of this study was to explore the possible correlation between OT and cortisol levels and mother-infant quality of bonding. OT and cortisol levels were measured using urine and saliva tests. Maternal bonding was measured by observation and a post-partum bonding questionnaire. Our results, using a group of 29 mother-infant healthy dyads, found no correlation between OT and the nurturing condition, but we found positive correlations between OT and the expressed feelings of mothers towards their infants, as well as a negative correlation between cortisol and less intense bonding feelings (feel afraid or resents infant). Understanding the association between mother and infant bonding interactions and induced hormones should be a valuable tool for paediatricians and psychologist in promoting behaviours that will benefit future adult behaviour and society at large.

Keywords: Oxytocin, cortisol, mother, infant bonding, effect.

<sup>\*</sup> ADDRESS FOR CORRESPONDENCE: Jana Olivova, Department of Psychology, Charles E. Schmidt College of Science, Florida Atlantic University, Boca Raton 33431, US. *E-mail address*: jolivova@fau.edu

#### 1. Introduction

Modern attachment theory states that an infant needs to develop a bonding relationship with at least one primary caregiver, especially with one or both parents, for the infant's successful social and emotional development, including the ability to cope with adversity (Ainsworth, 1963; Bowlby, 1958 and 1969; Bretherton, 1992). Early patterns of bonding and attachment will help shape (Hazan & Shaver, 1987) but not determine the expectation for later adult relationships. Specifically, motherinfant attachment (from initial bonding) is believed to play a major role in the way children will adapt to society. Understanding how mother-infant bonding occurs will be helpful in developing techniques to promote mother-child bonding and to teach future mothers how to positively interact with their children in situations of nurturing and adversity. This in turn may lead to more effective upbringing of children, who might become better-adapted and more productive members of society (Berlin, Zeanah & Lieberman, 2008). Hormones play an important part in body and mind development. Steroid hormones, such as sex hormones, have been associated with the brain 'behavioural wiring' or 'imprinting' in sexual preference, regardless of the genetic makeup of the foetus (Garcia-Falgueras & Swaab, 2010; Rice, Friberg & Sergey, 2012). Previous research has shown evidence for dyadic associations (i.e., participants' satisfaction and commitment were negatively related to their partners' levels of hormones, i.e., testosterone) and these levels associations were larger for women than men (Edelstein, van Anders, Chopik, Goldley & Wardecker, 2014).

More recently, there have been reports showing that the social hormone oxytocin (OT) and the stress hormone cortisol are likely related to dyadic attachment of mother—infant (Feldman, Weller, Zagoory-Sharon & Levine, 2007; Hardin, 2014). OT, a peptide hormone secreted by the brain, the hypothalamus and the pituitary gland, has been associated with a plethora of physiological and social behaviours, such as love attachment, post-traumatic stress disorder and more specifically mother—infant bonding (Bick, Dozier, Bernard, Simons & Grasso, 2013; Schneiderman, Zagoory-Sharon, Leckman & Feldman, 2012). The hormone is released upon touching and breastfeeding in mothers. Cortisol, a steroid hormone secreted by the adrenal glands and whose release is part of the hypothalamic—pituitary–adrenal (HPA) axis, is responsible for modulating behavioural and physiological responses to stress.

Despite advances in the field, few studies have been reported in the pre/post-partum HPA functioning and the oxytocinergic bonding system in mothers and their full-term infants as a consequence of pre-partum expectations as well as post-partum maternal care. In this study, we examined the effects of maternal pre- and post-hormones levels and full-term infant hormonal levels' impact on mother–infant interactions, and checked to see if there was a correlation between these two parameters. This knowledge will be useful to develop better nurturing techniques for expectant mothers. The objective of our study was to answer the following question: is it possible that cortisol and/or OT levels, as consequence of maternal pre-partum expectations and post-partum care, might play a role in the mother–infant quality of bonding? We hypothesised that there were correlations between these hormones' levels and the mother–infant bonding.

#### 2. Materials and methods

#### 2.1. Participants

Data were collected from 29 mother–infant dyads. Pregnant mothers between 29 and 38 weeks gestation was recruited from local hospitals, breastfeeding coalition meetings and through lactation consultants. Mothers recruited for the study did not use drugs or alcohol during the pregnancy and were not prescribed medications for mood disorders. They were screened for intention to breastfeed, for absence of depression and for perception of foetal bonding.

#### 2.1.1. Prenatal, neonatal, and 6-week and 3-month visits

The prenatal and neonatal visits took place in the participant-mothers' homes; the 6-week maternal interview was conducted via phone conversations; the 3-month visit was conducted in our Infant Development Lab at Florida Atlantic University. The feeding and interaction sessions and arm restraint procedures were recorded during the 3-month visit. A comprehensive overview of questionnaires, behavioural procedures and physiological measures were administered at each visit to make sure the participants were appropriately matched for our study. A post-partum bonding questionnaire (PBQ) was given at 3 months to ascertain the actual feeling of bonding or lack of it in mothers. See Appendix for questionnaire format.

#### 2.2. Hormone release stimulation

Stimulation of hormone release was induced by either nurturing conditions: a mother–infant play condition and breastfeeding condition for OT release or by a mother–infant stressful condition for cortisol release: the passive restraining of infant arms by the mother. In the play condition, the mother plays with the infant for 3 minutes and her level of nurturing and attentiveness to infant are coded. Verbal and physical displays of affection or lack of it, as well as mother–infant reciprocal interactions (mutuality) was the basis for coding. In the breastfeeding condition (timing for this condition will be determined at the mother's discretion), the mother's attentiveness to the child was coded for verbal/physical interaction as well. In the stress condition, the mother held down the infant's arms restraining his/her movement for 3 minutes while looking at him/her without showing emotion until the child cried (if applicable) and the quality of crying/fussing was coded. Cortisol and OT were measured by collecting mother's urine and infant saliva samples. Samples were collected over 4 consecutive days to establish average individual levels of OT.

# 2.2.1. Cortisol analysis

Salivary cortisol levels were measured at the pre-test phase (before the arm restraint procedure) and 20–25 minutes after the test phase, the elapsed time for cortisol levels to peak following a stimulus (Gunnar & Quevedo, 2007). By 3 months of age, infant salivary cortisol concentrations generally peaked in the morning and subsequently declined over the course of the day (Mantagos, Moustogiannis & Vagenakis, 1998); therefore, all saliva measurements were taken between 10:30 A.M. and 12 P.M. Cortisol was measured by collecting infant saliva samples using a Nalgene cryogenic vial (Thermo Scientific, NY, USA). The researcher allowed the infant to passively drool into the vial. A baseline saliva sample was collected as soon as the mother and infant arrived for the visit and again

20–25 minutes post-stressor. The samples were immediately placed in a –20°C freezer. Following the visit, samples were moved to an ultra-low temperature freezer at –80°C and subsequently assayed using a commercial cortisol EIA kit (Salimetrics, PA). The test required 25  $\mu$ l of saliva per determination and it has a lower sensitivity of 0.003 ug/dl. Samples were assessed for pH to be sure that they were within the normal range (6.5–7.5).

# 2.2.2. Oxytocin analysis

OT was measured by collecting maternal and infant urine. Samples were collected over 4 consecutive days to establish average individual levels of OT.

Mothers provided urine samples at the prenatal and 3-month visit. Mothers collected infant urine using paediatric urine bags (Thermo Fisher Scientific Inc., PA, USA) at the neonatal and 3-month visit. Mothers stored the samples in their freezers until they were done collecting samples, at which point the lab personnel transported the samples to a  $-80^{\circ}$ C freezer using coolers filled with ice. Urine samples were also stored at  $-80^{\circ}$ C. OT samples were assayed and analysed at the University of Wisconsin, Primate Lab by Dr. Toni Ziegler.

# 2.3. Experimental design

# 2.3.1. Behavioral recordings

A feeding interaction was video recorded at the newborn and 3-month visit, and then scored. Mothers were instructed to feed their infants as they normally would. The dyads were alone during feeding. The session concluded when the mother had finished breast or bottle-feeding the infant. A 5minute sampling of each dyad's feeding session was used for behavioural coding of the feeding session. The feeding session was coded for maternal touch, affect and vocalisations. Following the feeding session at the 3-month visit, mothers and infants engaged in a 3-minute play interaction. The mother and infant faced each other, with the infant placed in an infant seat. Mothers were instructed to interact with their infants as they would at home. Dyadic interactions were coded on a 40-second, second-by-second basis, for affect, touch and vocalisation. The gualities of interactions were assessed by combining affective coding into variables such as: high in affectionate care and low in affectionate care. Each behaviour of the dyad was coded separately. After the initial play session, mothers were asked to administer an arm restraint task to their infant. The infant was placed in an infant seat and the mother was instructed to hold down the arms of her infant for a 3-minute period. Mothers were then asked to maintain a neutral facial expression and remain silent during the procedure. Prior to and following this mild stressor, infant's saliva was collected through passive drooling for subsequent cortisol analysis.

# 2.3.2. Behavioural coding

Behavioural coding levels were assessed using scales described by Jones et al. (2004, adapted from Kuzela Stifter and Worobey, 1990; Stifter & Jain, 1996, respectively). The mother–infant measures of behaviours and maternal attention were touch, vocalisations and gaze. The scale used to code the mother–infant feeding session was also used for the mother–infant play session. The behaviours were

coded on a 7-point scale, with higher scores reflecting more optimal behaviours and lower scores reflecting suboptimal behaviours.

#### 2.3.3. Mother's self-assessment of bonding

A PBQ (Wittkowski, Wieck & Mann, 2007) was given at 3 months to ascertain the actual feeling of bonding or lack of it in mothers. See Appendix for questionnaire format. Correlations between the mother–infant bonding levels were obtained by plotting the behavioural data versus hormones levels. Pearson's correlation coefficients, which can give an indication of a strong correlation (Pearson's values close to 1) or positive or negative correlation (positive or negative coefficients, respectively), were obtained from these plots. The validity of the correlations was determined using the statistical null hypothesis (analysis of variance). The correlation was deemed significant if *p* values obtained are less than 0.05.

# 2.4. Determination of Pearson's coefficients

The data from the coding of behaviour were plotted using the software OBSERVER, a second-bysecond behavioural coding system, by two coders (JO) who were trained to use the programme to reliability (k = 0.83-0.93) and who were blind to the study's hypotheses to identify correlations and obtain significant Pearson correlation coefficients.

# 3. Results

In this study, we aimed to get a better understanding of the relationship between the hormones cortisol and OT and the quality of bonding between mother and infant. Specifically, to understand how the OT and cortisol changes over time, pre- and post-partum influenced the mother of attitude towards her infant in general.

OT versus maternal interaction				
	567 physical	OT increase-	OT increase-	
Mothers	+ 56 verbal	mothers	mothers	Mothers
567 physical + 56 verbal (composite variables)	Pearson's correlation	1	-0.228	-0.115
	Sig. (2-tailed)		0.262	0.672
	N	28	26	16
OT increase-mothers	Pearson's correlation	-0.228	1	0.252
	Sig. (2-tailed)	0.262		0.365
	N	26	27	15
OT increase-mothers	Pearson's correlation	-0.115	0.252	1
	Sig. (2-tailed)	0.672	0.365	

Table 1. OT increases and nurturing conditions

Variables were created by adding the highest coding scores (5, 6 and 7 in physical interaction and 5 and 6 in verbal interactions) and plotted versus OT increase in mothers. As OT in mothers increases, the maternal interaction appears to decrease (r = -0.228 and -0.115, p values are > 0.262 and 0.672,

respectively). Of 28 mothers, 26 had measured changes of OT values. There was no correlation, but rather the opposite trend than expected (r (26) = -0.228, p = 0.262). A subset of 16 mothers also showed no correlation (r (16) = -0.115, p = 0.672).

Table 2a. OT increase and maternal feelings (PBQ)				
Mothers	Anxiety about care	Reverse score PBQ 3 months		
(increased OT)	(Reverse 3PBQAC)	Total score		
Rejection/Anger (reverse 3 RAs) <sup>a</sup>	<i>r</i> =+ 0.647	<i>r</i> = + 0.758		
	(p = 0.000)	(p = 0.000)		
Participants	N = 29	N = 29		

Mothers' increased OT lead to positive correlations in three PBQ reversed parameters. Variables were rejection/anger (reverse 3 RAs), anxiety about care, 3-mo PBQ score.

<sup>a</sup>Here, reverse means reversed scale (1 = most and 7 =least).

A positive correlation between an increase in OT in mothers and a decrease in anxiety about care and rejection and anger as a consequence of OT increase was noted. Here, the parameters are plotted on a reverse scale; the higher the number from the PBQ questionnaire, the lower the feelings of anxiety and rejection of the mothers experienced.

Table 2b. OT time course versus rejection/anxiety (PBQ)				
Mothers	OT increase-mothers 1, prenatal	OT increase-mothers 2, postnatal		
Rejection/Anger (reverse 3RAs) <sup>a</sup>	<i>r</i> = + 0.148	<i>r</i> = + 0.218		
	( <i>p</i> = 0.472)	( <i>p</i> = 0.418)		
Participants	<i>N</i> = 26	<i>N</i> = 16		

A positive trend was observed between mothers with increased OT versus lower rejection and anger feelings.

<sup>a</sup>Here, reverse means reversed scale (1 = most and 7 = least).

Table 3a. Increase in infant's cortisol affects mother's behaviour				
Infants	Impaired bonding scale	Anxiety about care scale		
(increased cortisol)	of 3-mo PBQ	of 3-mo PBQ		
Cortisol After task	<i>r</i> = + 0.474	<i>r</i> = + 0.587		
	<i>p</i> = 0.014	<i>p</i> = 0.002		
Participants	<i>N</i> = 26	<i>N</i> = 26		

A positive correlation between increased infant cortisol and higher impaired bonding by mother as well as higher anxiety about care by mother was found (r (26) = 0.474 and 0.587; p = 0.014 and 0.002, respectively).

#### 4. Discussion

We expected to see more frequent and deeper levels of mother-infant bonding with increased amounts of OT in the mother-infant dyads, as well as in infants alone. But we did not find a positive correlation between the behaviour and the OT level. Our expectation was based on the fact that mothers might be pre-conditioned to bond with their infant as a result of her interest of getting

pregnant. Based on the PBQ questionnaire, the levels of higher OT in mothers correlated with lower feelings of anxiety and rejection for the baby. These lower feelings of anxiety and rejection decreased as the OT levels increased over time. However, our observations and coding of the observation and the increases in OT did not show a clear trend either way; therefore, we were unable to make any conclusions. The lack of a clear trend for this observation may be due to the small sample size of our study or it may be due to a lack of OT relationship to the specific mother–infant interactive behaviours chosen to study or that our OT measurements lack the minimum threshold for correlation. We expected that lower levels of bonding would be associated with cortisol increases. We confirmed this expectation based on the PBQ questionnaire: as the cortisol levels in the infant increased, higher levels of anxiety and rejection were observed by the mother. This is a very interesting result because the change in cortisol levels in the infant is related to the perceptions of the mother. We think that there is inverse relationship between a maternal perception of bond and infants that showed higher cortisol reactivity. Thus, infants who had higher cortisol reactivity were more likely to have mothers that viewed them infant as more difficult to handle.

As a future project, the effect of mother–infant bonding on infant brain maturation should be studied to see if there is a change as a result of prenatal (mother) and postnatal (infant) hormonal levels, the latter being induced by postnatal mother–infant interactions.

It is possible that brain maturation occurs solely as a consequence of hormonal levels. This is difficult to ascertain with non-interventional studies. Only so-called natural experiments, where infants would accidentally be exposed to large OT or cortisol levels and no maternal nurturing, could shed light on this hypothesis. Since our study takes snapshots of data points, we might find that mothers who appear nurturing in the lab are not nurturing in the privacy of their homes. This could be corrected using a different type of screening method or by the use of a control group of mothers who would allow a 24 hours video monitoring of their in-home behaviour.

We can conclude that a strong mother–infant bonding (nurture) is just as important as hormones levels themselves (nature). The nurturing part of the child rearing should have an important role in overriding or modulating the natural hormonal levels effects. Thus, we expected that the PBQ would shed light on our latter expectation behaviour or condition outside the lab in their daily interactions with their infants and, therefore, might have infants whose brain maturation happens at an earlier time. Follow-up questionnaires might be helpful in determining if this latter hypothesis is correct.

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# Appendix

# **PBQ** Questionnaire

- 1. I feel close to my baby.
- 2. I wish the old days when I had no baby would come back.
- 3. I feel distant from my baby.
- 4. I regret having this baby.
- 5. The baby does not seem to be mine.
- 6. The baby winds me up.
- 7. My baby irritates me.
- 8. I feel happy when my baby smiles or laughs.
- 9. I love my baby to bits.
- 10. I enjoy playing with my baby.
- 11. My baby cries too often.
- 12. I feel trapped as a mother.
- 13. I feel angry with my baby.
- 14. I resent my baby.
- 15. My baby makes me anxious.
- 16. I wish my baby would sometimes go always.
- 17. My baby is the most beautiful baby in the world.
- 18. I am afraid of my baby.
- 19. My baby annoys me.
- 20. I feel confident when changing my baby.
- 21. I feel the only solution is for someone else to look after my baby.