TITLE: THE EFFECT OF INTRANASAL OXYTOCIN ON THE NEURAL PROCESS OF CHILD-RELATED SOCIAL SIGNALS ON YOUNG MOTHERS

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ABSTRACT:

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Background: Over the past few decades, oxytocin (OT) has been identified as a neural hormone playing positive roles in parental bonding, sexual behaviours, and social affiliation behaviours. Additionally, OT has shown to be correlated with parental sensitivity, parenting contact, and parent-child synchrony, thus helping to strengthen the parent-child bonding. Therefore, the use of OT as an intervention of harsh and difficult parenting such as maternal depression or parent-child bonding disorders has attracted wide attention from academia and research.

Objectives: The thesis study primarily aimed at investigating whether intranasal administration of OT affected the neurocognitive process in young mothers perceiving infant's facial emotional stimuli via the Event-related Potentials (ERPs), i.e. a neural technique used to investigate brain functioning . Subsequently, the secondary objective was to examine whether the subjective perception of bonding in mothers got influenced by OT via two questionnaires.

Data and methods: The thesis study was a sub-set of the project study conducted by University Hospital of Tampere (TAYS), from which two different types of data were extracted and analysed : the ERPs (three components: N170, EPN and LPP) and two modified questionnaires (i.e. Postpartum Questionnaire (PBQ) and Experiences of Close Relationship-Relationship Structure Questionnaire (ECR-RS)). The thesis study was conducted in a placebo-controlled, double-blind, within-subjects design with 52 participants. They were asked to visit one laboratory two times with a one-month interval in order to perform the ERP task and fill in two

questionnaires. Using the ERP data for the primary objective, the N170 and posterior EPN components were analyzed with a 2 x 2 x 2 x 2 repeated-measures analysis of variance (ANOVA) with *Face* (Infant vs. Adult), *Emotion* (Happy vs. Sad/distressed), *Condition* (OT, vs. PL), and *Hemisphere* (left vs. right) as within-subjects factors. Likewise, the LPP data were analyzed with 2 x 2 x 2 ANOVA with the factors *Age, Emotion and Condition*. In contrast, the questionnaire data was analysed through a paired sample *t*-test to compare the results of the questionnaires between OT and placebo condition.

Results and Discussion: When analyzing interactions among variables in all three ERP components, OT was not found to significantly affect the neurocognitive process in young mothers (p-values= 0.97, 0.13 and 0.46 for N170, EPN and LPP, respectively). Interestingly, then interaction *Face x Condition* in the N170 component was found to be marginally significant (F (1, 37) =3.30, effect size=0.08, p-value=0.07), indicating that there was a difference in the effect of OT on the social-emotional mothers' perception. However, this effect was either faintly supported by the present analysis or very minute if the effect did exist. Besides, only in the N170 component, the main effect of *Condition* appeared significantly (F (1, 37) =15.11, effect size=0.16, p-value=0.01), which indicated the OT held larger negativity than placebo (-6.65±0.50µV and -6.34±0.49µV, respectively). Contrary to expectations, the results of two questionnaires yielded no significant difference in questionnaires' scores of the mothers when receiving either OT or placebo (p-value=0.6, 0.82 and 0.34 for the PBQ, *Avoidance* ,and *Anxiety* scores of ECR-RS, respectively). The study contributes to the current evidence of the pro-social effects of OT, which has shown to be inconsistent and incoherent. Therefore, successive studies with larger population could be conducted to increase the reliability of the results. Moreover, the effect of genetic factors and epigenetics should be also taken into account in further studies since literature evidence suggests individual variations in OT may contribute to OT responses.

Keywords: OT, double-blind within-subject design, Event-Related Potentials, young mothers, infant's face stimuli, Postpartum Questionnaire, Experiences of Close Relationship-Relationship Structure Questionnaire.

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Contents

ABBREVIATION:

ERP: Event-related Potential

ERPs: Event-related Potentials

EEG: Electroencephalogram

EPN: Early Posterior Negativity

N170: Negative 170

LPP: Late Positive Potential

OT: Oxytocin

PL: Placebo

PBQ: Postpartum Bonding Questionnaire

ECR-RS: Experiences of Close Relationship-Relationship Structure

IU: International Units

mU: milliunit

PVN: ParaVentricular Nucleus

SON: SupraOptic Nucleus

fMRI: Functional Magnetic Resonance Imaging

SD: Standard Deviation

INTRODUCTION

Since past decades, there have been such numerous publications and research studies supporting the hypothesis that intranasal oxytocin (OT) plays a role in promoting the processing of social stimuli, thus regulating socially emotional behaviours including trust, attachment memory, fidelity, and even anxiety. The OT is thought to be positively correlated to social behaviours including social cognition and social interaction, emotional process, attachment and reduced stereotype behaviour(Guastella & MacLeod, 2012). Over the past few decades, the role of OT in promoting bonding relationships, enhancement of peer recognition, which were proven in animal studies across a variety of mammalian species(Nave, Camerer, & McCullough, 2015).

Additionally, OT has been well recognized to play a significant role in reproductive function such as mating (Frederique, Serge, Kathleen, & Guertin, 2013), in the female reproductive process (e.g. facilitating parturition and milk ejection during lactation) and in promotion of maternal caregiving behaviours (Carter, 2014). Synthetic OT (Pitocin®, Syntocinon®) has been widely used to induce or augment labour and postpartum care in modern obstetrics (Bell, Erickson, & Carter, 2014). More than a half of mothers who gave birth in the USA are administered synthetic OT during their labour (J. A. Martin et al., 2012). However, the effectiveness of given doses of OT varies across individuals, ranging from 1 milliunit per minute (mU/min) and greater than 40 mU/min. Furthermore, beyond the common use in a clinical setting, the biological and behavioural effects of synthetic OT are still mysterious to scientists (Bell et al., 2014).

In primates including animal, OT furthermore facilitates the reproductive process, starting from pushing babies from the uterus, stimulating milk production and forming the mother-child bonding. Obviously, newborns depend on their mother milk for their first few months and mothers also form the bonding with their babies right after birth, in the period which is crucial for babies survival and development. In addition, parental care and support are significantly important for physical development and mental health of babies. Numerous

studies in animals (e.g. rodents) and lactating mothers have demonstrated that OT is able to modulate the stress response and defensive behaviours caused by separating from mothers, thus support the growth and health of babies (Keverne, 2006).

Particularly, the use of nasal spray for administering OT has then become a standard method of behavioural research (Evans, Dal Monte, Noble, & Averbeck, 2014). During recent years, there have been several experiments using intranasal administration of OT in order to understand human perceptions, emotions, and behaviours. Due to proposed beneficial effects of OT, there is an interest in using OT as therapeutic interventions for social-related difficulties such as postnatal depression, mother-child attaching difficulties or recently child autism has increased dramatically (Guastella & MacLeod, 2012; Liu, McErlean, & Dadds, 2012; Yatawara, Einfeld, Hickie, Davenport, & Guastella, 2016).

Despite numerous findings of OT benefits and our basic understandings of OT on social behaviours, there is still limited research about casual effects of intranasal OT on a social-emotional process in mothers. Therefore, the Tampere University Hospital (i.e. TAYS) proposed and conducted one study project called "Effects of maternal OT on social information processing in mothers". The aim was to investigate if the neurocognitive processes in perceiving children's facial information are influenced by OT. The projected has been registered on EU Clinical Trials database with the EudraCT number 2014-003728-39.

Certainly, with regard to study design, the study project was conducted in a placebo-controlled, double-blind and within-subjects setting. The result from the project would provide empirical evidence to consider the feasibility of intranasal OT intervention for early child-mother interaction. Plentiful evidence suggesting that child development is largely dependent by parent–child interaction. Thus a secure parent–child attachment can predict positively associated with various outcomes of child development (De Falco et al., 2014). Therefore, the thesis study was chosen to analyze partially data obtained from that project study with the aim of investigating relationships between OT's effects and the process of social information in young mothers, using only Event-Related Potential (ERP) and questionnaire data.

CHAPTER 1: BACKGROUND OF THE STUDY

The thesis study focuses on examining the effects of intranasal OT (vs. placebo) administration to mothers on their neural and behavioural responsivity to children's emotional signals. In an effort to provide background information of this thesis study, the literature review introduces firstly the chemical and physiological property of OT, then the roles of OT in pro-social behaviours in human beings, in labour and lactation and in parenting/ mothering behaviours, and eventually, the ERP technique and its application in neurological research.

1.1. Anatomical and physiological characteristics of OT

Oxytocin has a chemical anatomy of nine-amino-acid units functioning in bodies both peripherally as a hormone and centrally as a neurotransmitter (Evans et al., 2014). It has a chemical shape of six amino-acid-ring and three-amino-acid tail. Some of its functional characteristics can be explained by its dynamic biological properties of sulfur bonds, which both form the ring and allow OT links either permanently or temperately to other five chemicals (W. L. Martin & Carter, 2013). It is synthesized by the magnocellular and parvocellular neurons of the paraventricular nucleus (PVN) and supraoptic nucleus (SON) of the hypothalamus, and then released into the bloodstream by the posterior pituitary gland (Carter, 2014). Because PVN is a major site of convergence and integration of neural communication especially for stress and adaptive responses, thus OT may be co-released as a stress-hormone in order to respond adaptively to positive-or-negative challenges (Neumann & Landgraf, 2012). Other cells can release OT including neuronal soma, axons and dendrites. OT then can be modulated widely in the nervous system and carried through neural tissue (Carter, 2014).

Generally, OT can be found in all mammalian species including human beings but its level varies across species. Furthermore, different human individuals have different OT levels and the difference is due to individual traits, individual social behaviours and even socially associated disorders such as schizophrenia and autism (Gouin et al., 2010).

1.2. OT in human affiliation

The first possible "pro-social" role for OT was documented in one study nearly four decades ago in a behavioural study using virgin female rats (Pedersen & Prange, 1979). Successive studies indicated that the release of OT could impact pair bonding in the monogamous female prairie vole, compared with olfactory signals (Williams, Insel, Harbaugh, & Carter, 1994). The results have inspired following researchers to seek for corresponding effects in human beings. Therefore, a large number of published studies since last decade have supported an effect of OT in enhancing the processing of social stimuli, thus promoting human affiliation and affiliation-related behaviours. Trust, the absence of fears, and maintenance of healthy interactions with the environment are fundamental factors of an overall sense of well-being (IsHak, Kahloon, & Fakhry, 2011).

Moreover, OT inhaling has been found to be associated with increased trust. In a test of the financial investment, a group of male participants (n= 58) receiving intranasal OT 50 min prior to test displayed a higher level of trust (i.e. higher money transfer amount per unit per investor) than the group receiving placebo. Even when the participants were aware of the increased risk, identical money transfer was still recorded. The effect of OT on trust is ,therefore, suggested to be unrelated to increased risk-taking behaviours (Kosfeld, Heinrichs, Zak, Fischbacher, & Fehr, 2005).

Furthermore, OT has been showed to shape neural circuitry of trust and trust adaption in human beings. In one trust-game, OT-received individuals (n=49) were found to be persistent in trusting behaviours even after several betrayals while the placebo group lost their trusting behaviours(Baumgartner, Heinrichs, Vonlanthen, Fischbacher, & Fehr, 2008). OT exerts this effect through modulating amygdala activities. Amygdala is an almond-shaped region in the temporal lobe, which functions in mediate fear, trust, anxiety and social recognition (Ressler, 2010). In the trust game, the OT-treated group showed reduced the activation of amygdala midbrain and the striatum region on fMRI. Therefore, amygdala deactivation can explain why OT enhances trust behaviours (Ressler, 2010).

OT also plays role in reducing fear via suppressing amygdala activation. In a report by Krisch (2005), OT infusion attenuated the sympathetic behavioural expression of fear in response to fearful and angry faces (Kirsch et al., 2005). Another study using a single dose of intranasal OT on healthy males (n=27) indicated an increase of the participant's ability to recognize fear, compared to the placebo group (Fischer-Shofty, Shamay-Tsoory, Harari, & Levkovitz, 2010). The finding of the suppressed amygdala activation under OT supports the hypothesis, which the reduced amygdala activation to fearful faces is linked to decrease social fear, increase sociability and enhance trust (Kirsch et al., 2005).

1.3. OT in sexual behaviours

There has been abundant evidence that OT may enhance the fidelity of monogamous relationship. In an early stage of a romantic relationship, the high level of plasma OT was used to predict if the attached relationship could sustain more than six months (Feldman, Gordon, Schneiderman, Weisman, & Zagoory-Sharon, 2010). In a study of human couple interaction, a single dose of OT delivered prior to standardized couple conflict discussion resulted in significantly reduced anxiety levels in couples as indicated by the subjective evaluation and salivary cortisol levels. Additionally, the OT-received group displayed elevated positive communicative behaviours and lessened negative attitudes during the conflict sessions (Ditzen et al., 2009). Interestingly, OT administration caused males in a monogamous relationship, but not singles one, to behave cautiously in front of other females (i.e. kept their distance from attractive females or approached more slowly to attractive ladies). Therefore, both intranasal OT and the high basal level of OT promoted the attached relationships to a partner and reduced negative behaviours during couple conflicts (Scheele et al., 2012).

1.4. OT in labour and lactation

One of the primarily well-known roles of OT is in labour and lactation. Synthetic OT such as Pitocin®, Syntocinon® is frequently used to facilitate birth induction via stimulating uterine smooth muscle contractility, then transiting to augmentation and the third stage. It has been

estimated that the induction rate has been more than doubled from 1990 (10%) to 2010 (23%), according to the U.S. Centers for Disease Control and Prevention Vital Statistics report(Bell et al., 2014).

During lactation, an increase of OT pulses in the brain results in the more secreted hormone in blood circulation. Via measuring salivary OT in lactating women, OT has been recorded to be elevated prior to feeding as mothers prepare to feed babies (White-Traut et al., 2009). Meanwhile, the raised OT level has altered the stress-linked response system; therefore, lactating mothers are showed to less react to stressors and present less anxiety-like behaviours than non-lactating females. The response of the increased OT level is proposed to be the protective mechanism against heavily stressed conditions (Bell et al., 2014).

Naturally, the transition to motherhood is linked to a drastic hormonal shift to cope with new stressors (i.e. physical pain, lactation and attachment) which occur during physiologic birth (Bell et al., 2014). The significant rise of endogenous OT during the birth perinatal period is considered an adaptive respond and protective mechanism in buffering stress hormones(Carter, Altemus, & Chrousos, 2011). Furthermore, the increased level of the peripheral OT released during birth, breastfeeding, and skin-on-skin mother-infant contacts has been recorded to be proportionally correlated to better maternal responsiveness, reduced maternal stress and enhanced mother-infant bonding (Carter, 1998). High level of endogenous OT in the first trimester can act as a predictor of the volume of maternal behaviours (Altemus et al., 2001).

1.5. OT in parental bonding and parental attachment

Plentiful research on the associations between OT and parenting has shown that parental sensitivity responding to infant signals and parental behaviours are crucial for the social-emotional development of children since it forms secure infant attachments to parents (De Wolff & van Ijzendoorn, 1997). Therefore, any difficulties in motherhood transition can lead to dysregulated stress reactivity in mothers and poor mother-infant attachments, which then gives impacts on later child development (Bell et al., 2014). OT has been implicated to have a

foundational role in promoting sensitive caregiving behaviours and concrete mother-infant bonding behaviours such as gazes, attachment-related thoughts, affectionate touch and vocalizations (Feldman, Weller, Zagoory-Sharon, & Levine, 2007; Galbally, Lewis, Ijzendoorn, & Permezel, 2011). Additionally, OT has been described to be correlated with the amount of stimulatory paternal behaviours such as tactile stimulation, proprioceptive contacts and object presentations(Gordon, Zagoory-Sharon, Leckman, & Feldman, 2010).

1.6 OT in mothering behaviours.

Numerous animal studies have demonstrated the role of OT in mediating the initiation of mothering behaviours. Research on non-human species has provided strong evidence on functional maternal behaviours. For example, OT clearly mediated maternal behaviours in rats (Pedersen, Caldwell, Walker, Ayers, & Mason, 1994) while synthetic OT promoted maternal acceptance of alien lambs in ewes (Keverne & Kendrick, 1992). In prairie vole which is socially monogamous species like human beings, OT was found to be an important regulator for juvenile female expressing alloparental care toward non-biological pups (Olazábal & Young, 2006). Optimal maternal behaviours can be modified in animal studies by OT antagonists or central injections of synthetic OT (Bell et al., 2014). Studies on animal models can shed the light for understanding the role of OT in mothering behaviours in human beings.

In addition to animal studies, neurological research on human beings has implicated roles of OT in processes and formation of maternal bonding and mothering behaviours. Optimal maternal behaviours can include exclusively infant-eye contacts, affectionate touch and thoughts, synchronous mother-infant interactions, and sensitivity to infant cues and vocalizations (Feldman et al., 2007). Association between atypical peripheral OT and lower optimal mother behaviours has been extensively studied. Some culprits behind less mothering behaviours can be assigned such as genetic variations (i.e., risk alleles), and decreased central binding of the OT receptor (Bell et al., 2014). Furthermore, the role of OT also has related to female's affiliative experiences throughout her lives including her own parents, her spouse and babies (Feldman, Gordon, & Zagoory-Sharon, 2011). One study showed that women who gave

birth with the help of a doula (i.e. a woman gives support, advice and help during pregnancy and birth) would need less painkillers and would have better long-term outcomes such as reduced maternal depression, less problems with lactation and maternal social interactions (Landry, McGrath, Kennell, Martin, & Steelman, 1998). Similarly, in a study in which maternal OT levels and outcomes were monitored four days after giving birth and followed for two months, those women with high level of OT reported subjectively less struggles in coping with depressive symptoms (Uvnäs-Mobcrg, Widström, Nissen, & Björvell, 1990). Therefore, the presence of a doula was proposed to stimulate the OT release and to be associated with successive neuro-chemical changes, thus making those women less likely to have problems with mother-infant interaction and breastfeeding than those with caesarian deliveries (Uvnäs-Moberg, 1998).

1.7 The ERP technique and applications on study of adults' responses to child 's signals

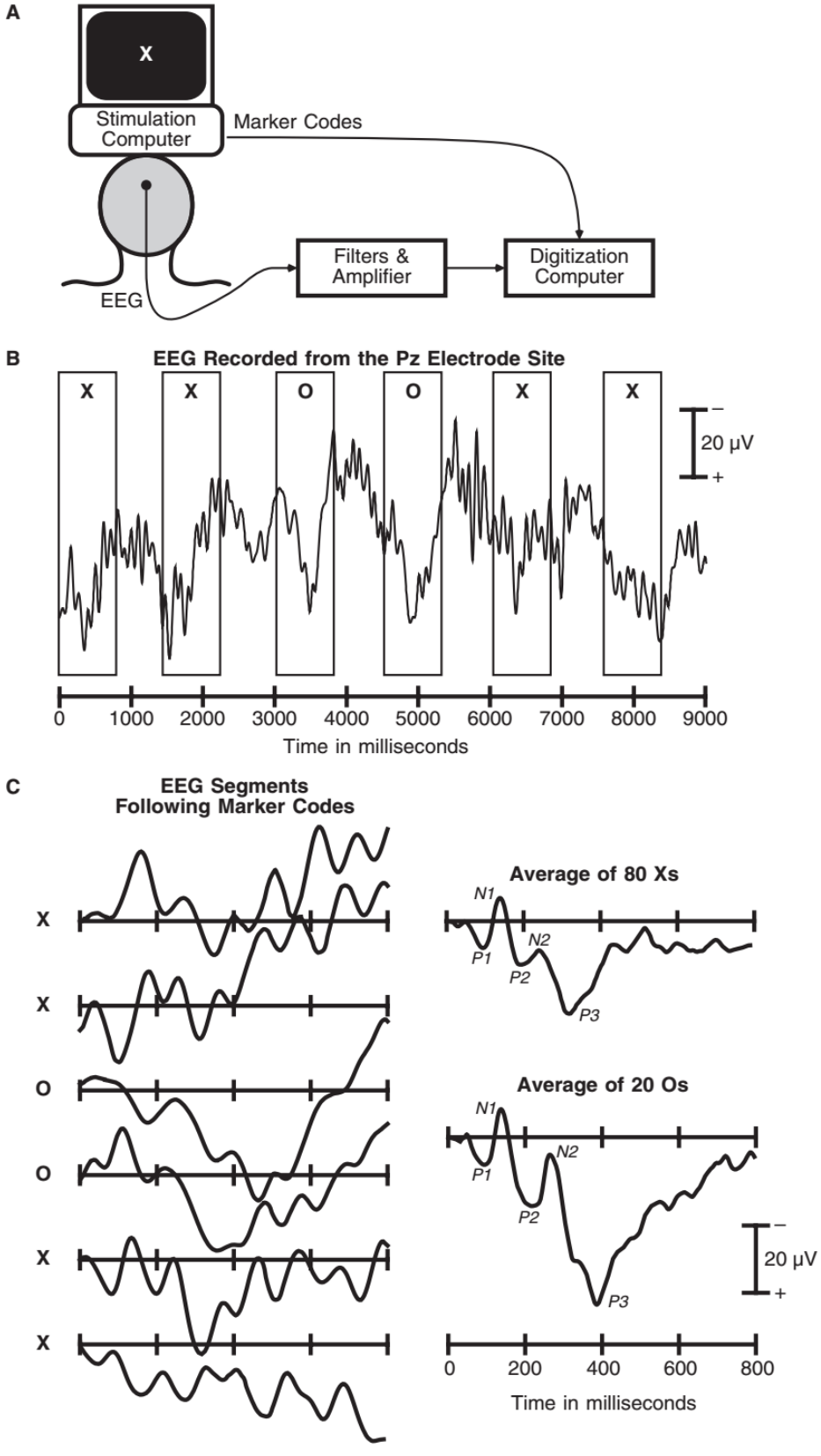
The ERP is a neuropsychiatric research method using "very small voltages generated in the brain structures in response to specific events or stimuli" (Sur & Sinha, 2009). It consists of millisecond-to-millisecond records of cortical responses related to neural information processing. Actually, Event-related Potentials (ERPs) are electroencephalogram (EEG) changes, which are recorded and displayed as small voltage changes in the brain structure toward specific events/stimuli via time blocking. It is used as a safe and noninvasive tool to study psychophysiological correlates related mental processes and neural activities (Thigpen & Keil, 2017)

One advantage of ERPs is its polarity's (positive- and negative-going voltages) position along the waveform, which means that it allows to examine stimulus processing at various functional stages. In a mother-child relationship, when a mother' brain selectively responses to her child's face, it provides hints on her ability to take actions toward her child under different circumstances, thus ensuring child survival and healthy development (Grasso, Moser, Dozier, & Simons, 2009). Responses of parents, especially mothers to infant cues have been a topic of ample neuropsychological research and it suggests that those responses must be quick and

intuitive (Rutherford et al., 2017). Therefore, it requires a time-recording sensitive neural technique which can track OT modulation in process of cognition, attention and perception toward infant cues (Hajcak, Dunning, & Foti, 2009). ERPs provide an excellent resolution to investigate the variation of stage of the neural process in parents and non-parents (Maupin, Hayes, Mayes, & Rutherford, 2015).

Especially in human beings, ERPs can be classified into two categories depending on the length of time after stimuli presentation. The early waveforms are termed "sensory" or "exogenous", elicited roughly the first 100 milliseconds (ms) after stimulus onset. It depends largely on physical parameters of stimuli. The latter waveforms (recorded after 100ms) are labelled "cognitive" or "endogenous", which are employed to examine the information process of the participants because they reflect the manner in which participant deal with stimuli.

Additionally, the waveform is usually described based on its latency and its amplitude (Sur & Sinha, 2009). The ERP waveforms consist of a sequence of negative and positive voltages. The waveform of ERPs is also called "component", "peak" or "wave". Commonly, letter P and N are used to indicated positive-going and negative-going peaks, respectively; the numbers behind letters such as 300 (of P300) or 170 (of N170) imply either the position of the peak in the wavelength or its precise latency. Conventionally, a negative-going waveform was plotted upward while a positive-going was downward. The flow of information in the neural activity is reflected by the sequence of ERP waveforms (Luck, 2014). Figure 1 simplifies the EEG set-up (A), signal recordings from electrodes (B) and waveform's read-out (C)


A
X
Stimulation Computer
Marker Codes
Filters & Amplifier
Digitization Computer
EEG
B
EEG Recorded from the Pz Electrode Site
X
X
O
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20 µV
0
1000
2000
3000
4000
5000
6000
7000
8000
9000
Time in milliseconds
C
EEG Segments Following Marker Codes
X
X
O
O
X
X
Average of 80 Xs
N1
P1
N2
P2
P3
Average of 20 Os
N1
P1
N2
P2
P3
20 µV
0
200
400
600
800
Time in milliseconds

(credited from (Luck, 2014))

In this thesis study, we examine three components of ERPs: N170, EPN and LPP. Firstly, the N170 component is the most important electrophysiological index of face processing which was first reported in the mid-1990s (Bentin, Allison, Puce, Perez, & McCarthy, 1996). Now it has become one of the most widely used ERP markers of face perception. There have been several studies used the N170 component to investigate the time length and functional properties of different aspects of face processing in the human brain (Eimer, 2011). It is a negative ERP component occurring approximately 170 ms after the stimulus presentation and usually with a lateralization towards the right hemisphere (Hileman, Henderson, Mundy, Newell, & Jaime, 2011). A meta-analysis by Hinojosa and colleagues (2015) concluded that the N170 was an early face-sensitive ERP component, which supported the proposal for an integrated mechanism in process of identity and protection. In addition, the facial sensitivity differs in various emotions, in which angry, fear and happy faces trigger the largest N170 amplitudes (Hinojosa, Mercado, & Carretié, 2015).

Secondly, Early Posterior Negativity or EPN is a negative-spreading wave which is arising around 220-320 ms after the stimulus presentation and reflecting selective visual attention toward emotional stimuli. (Schupp, Flaisch, Stockburger, & Junghöfer, 2006). Thirdly, Late Positive Potentials or LPP is collected by Pz and CPz electrodes and evoked by emotionally engaging stimuli. Allocation of attention to emotional or motivational stimuli is thought to be reflected by LPP (Hajcak et al., 2009). This component is characterized by an amplitude enhancement by either pleasant or unpleasant stimuli (Schupp et al., 2006). In this study, LPP starts from 300-600ms after picture presentation.

Overall, the OT, normally called “love hormone”, has been attracted considerable attention from scholars and researchers in fields of neuro-behavioural and pyscho-social sciences, especially parenting and parental behaviours. Besides, the use of the ERP technique in the field of neurology and psychiatry/psychology has become widespread, thus continuously providing new insights of human brain processes. In this thesis study, the ERP technique is applied to

investigate the effect of OT on mothers' neural processing when seeing infants' faces, compared with seeing adults' faces, thus shedding the light of understanding the information process of mothers in mother-child relationships. The next chapter, the objectives of the thesis study is mentioned in detail, corresponding to two different datasets.

CHAPTER 2: AIMS OF THE STUDY

The thesis study was a subset of a project study by TAYS, which was looking at the relationship between OT's effect and the process of social information in mothers.

Therefore, along with that project study, the aim of the thesis study was to examine if the neurocognitive process in mothers were influenced by OT in perceiving child's facial signals. Via a placebo-controlled, double-blind, within-subject design, the study research questions include:

(1) What is the effect of intranasal OT to mothers on their neural and behavioural responses to child's signal as compared with placebo?
(2) Does OT influence the subjective bonding perceptions of mothers of their children in the child-mother relationship? Does OT enhance secure/insecure attachments of mothers when thinking of their own mothers?

In order to answer these research questions, sufficient materials and suitable methods must be carefully evaluated and chosen, which is presented in the next chapter.

CHAPTER 3: MATERIALS AND METHODS

Because the project study (certainly including my thesis study) is an interventional study, thus it is indispensable to describe its study participants, the strict inclusive/exclusive criteria, the recruitment process, the experimental procedure, and the study drug's preparation. In addition, proposed hypotheses and corresponding data analyses are mentioned in detail. Moreover, the ethical aspect and the ethics approval, as well as planned statistical methods are presented lately.

3.1 Study participants and recruitments

There were fifty-two healthy female participants included in the project study. Subjects were contacted via the Population Registration Center (Väestörekisterikeskus) and received invitation letters of participating the study. Participants were invited to visit the laboratory twice with a one-month interval. The chance receiving OT was always 50% among participants.

The inclusion criteria were healthy female subjects with age ranged from 20-40 years old and mothers of young children. Because OT could stimulate lactation, thus the participants might become aware of the received substance. In order to maintain the effectiveness of the double-blind design, only mothers, therefore, who did not or had stopped breastfed at least one month before the first planed laboratory visit, were included. Approximately, more than half of Finnish women stopped breastfeeding their babies by the age of six or seven months, thus mothers of infants aged from 12 to 14 months were recruited to join the study (Uusitalo, Nyberg, Pelkonen, Sarlio-Lähteenkorva, & Hakulinen-Viitanen, 2012).

The exclusion criteria were (1) breastfeeding, (2) any known neurological problems , (3)visual, and auditory impairments (4) use of medications (except oral contraceptives) (5) drug or alcohol abuse (6) psychiatric disorders (7) nasal disease or obstruction (8) smoking (9) pregnancy and (10) cardiovascular diseases. Within 24 hours before the visit, mothers were

instructed not to consume alcohol and extreme physical activities. Coffee could not be consumed 4h before the test.

3.2 The procedure of the ERP experiment and the questionnaire.

The whole project study (EudraCT number: 2014-003728-39, ETL-code R14157M) was conducted in the double-blind, placebo-controlled, within-subjects design with the four-week interval between two laboratory sessions. Certainly, the informed consents had been delivered to each subject before entering any part of experiments. The project study contained two EEG tasks (i.e. one for ERPs and the other for asymmetry of EEG alpha power distribution) one non-EEG task and two questionnaires. Participants were carefully explained about all procedures before signing informed consents. In the thesis study, only data of ERPs and questionnaires were chosen to describe and analyze because it was fairly complicated and extensive to include all analyses of the whole project study in a master thesis.

Prior to undertaking the first laboratory session, information about pregnancy, medications, visual impairments and current nasal diseases and obstruction were collected from participants via a pre-task questionnaire. Each participant received one puff of the substance (either OT or placebo) in each nostril via an LMA MAD Nasal™ atomization device. Half of the mothers were randomized to receive OT in the first visit and placebo in the second visit while the other half received the substance in the reversed order. The randomization order was designed and conducted by one pharmacist of the TAYS hospital. At the end of the second visit, the awareness check was completed by each participant to confirm that the possibility of correctly guessing which substance(s) received was just by chance, $t(48)=0.14$, $p=0.89$.

In the experiments, the ERP data were collected approximately 55 minutes after intranasal spray administration. This time fell within the time-window, which was showed to be associated with significantly increased oxytocin levels in saliva following intranasal oxytocin administration (Huffmeijer et al., 2012). In addition, the time allowed to sustain the increases

of oxytocin-induced activation in brain areas important for social cognition and emotion processing(Paloyelis et al., 2016).

The ERPs were collected via 64 active EEG electrodes mounted in an elastic cap (actiCAP) on the participant's head. The signals were amplified by a QuickAmp amplifier (Brain Product GmbH, Munich, Germany) with a 1000-Hz sampling rate. The ERPs were used to measure the brain activity in response to stimuli of happy or sad/distressed infant or adult faces. The purpose of this task was to test whether the intranasal OT enhanced the recognition and attention–related ERP responses to child emotional stimuli.

Regarding photographic stimuli, the images of faces were presented on 19-inch screen with a viewing distance of 70-cm. The source of infant faces was from Baylor College of Medicine (Strathearn, Li, Fonagy, & Montague, 2008) and of adult faces from the Karolinska Direct Emotional Faces database (http://www.emotionlab.se/resources/kdef). There were 6 individual faces in total (3 females and 3 males) for both adult and infant face stimuli in the ERPs. All images were cropped external contours, converted into grayscale and presented in a gray background. The images of infant face were extracted from videotaped situations in which infants were given favorite toys, thus expressing happy moments whilst sad/distressed emotions elicited by leaving infants alone for a short time. The average age of infants was approximately seven months. In adult face, the sad emotions were selected so that they were closely resembled ones in infancy (Camras & Shutter, 2010).

In the ERP task, a series of unfamiliar pictures of either infants or adults expressing distressed/sad and happy faces were shown randomly on the screen. On the trial, each member of two arms (OT vs. placebo) had to press a button to decide whether the expression of the face was happy or sad regardless of stimulus ages (infant vs. adults). The participant was instructed to keep their eye on the face while the face's image was still visible on the screen until deciding to press buttons to indicate positive or negative emotions. Each face was presented on the screen for 700 ms followed by a response prompt until the participant's response. A short blank interval of 750 ms between two facial images was presented on the screen. With 60 times,

repetition of 2 stimuli (adult/infant) x2 stimulus (sad/happy), a total of 240 trials were run in a 12-minute task. Each individual model with the corresponding emotion was repeated 10 times. The order of response button was balanced across two visits.

In addition to attending laboratory tasks, the mother was asked to fill out two brief questionnaires, one for assessing bonding to the infant (i.e. PBQ questionnaire) and the other for attachment representation toward the mother's own parents (i.e. ECR-RS) at the end of each session. The PBQ questionnaire includes 11-item questions, which was the impaired bonding subscale of the Postpartum Bonding Questionnaire suggested by Brockington (Brockington, Fraser, & Wilson, 2006) . One item was originally excludes from the PBQ, that was " I sent my baby back" because it was too extreme for non-clinical population. The ECR-RS assessed 9-item questions, which belong to the mother-relationship section of Experiences in Close Relationship-Relationship structure questionnaire by Frayley (Fraley, Heffernan, Vicary, & Brumbaugh, 2011).

3.3. Administration of study drug

In the project study, the chosen investigational product was Syntocinon® (ATC code: H01BB02) nasal spray. This is a product of Novartis and distributed in Finland by Tamro (product number. 2128593). Each Syntocinon® bottle contains 40 International Unit (IU) of OT (4 IU/dose) as an active ingredient and 0.4 mg methylis and 0.2 mg propylis parahydroxybenzoas as conservation substances. All the investigational and placebo (saline) spray syringes were used only once. The Tampere University Hospital Pharmacy was responsible for preparation of spray syringes used to ensure the double-blind manner of the study (i.e., marked by the participants and visit number codes). Each participant received one puff, which is equivalent to 0.3 ml of substance, for each nostril (i.e. in a total of 0.6 ml). An LMA MAD Nasal ™ atomization device (http://www.lmaco.com/home) was used to deliver 24 IU of OT or placebo to participants

3.4 Testing hypotheses and data analyses

3.4.1 Records of Event-related brain potentials.

Our hypothesis was that intranasal OT promotes the recognition of the ERPs and attention-related ERP responses to a child' s emotional stimuli, especially to the signal of distress.

For the ERPs, the continuous EEG signals were first filtered then corrected for ocular artefacts (i.e. eye blinks and horizontal eye movements) and residual artefacts. The N170 components were extracted within a time window of 120-220 ms after picture representations and collected from a set of electrodes over left and right posterior locations (i.e. P7, PO7, PO9,TP9 for the left side, and P8, PO8, PO10, TP10 for right side). The EPN response was quantified as the mean activity within 220-320ms in the same electrodes as in the N170 component while the LPP response was within 300-600ms over the parietal midline electrodes Pz and CPz.

3.4.2 Assessment of mother-infant bonding via questionnaire data

As mentioned the experiment procedure, the mothers completed two brief questionnaires, i.e. PBQ and ECR-RS at the end of the laboratory visit. Regarding the impaired bonding subscale of PBQ, it was hypothesized that OT could increase the mothers' perception of bonding toward her own child. For ECR-RS, the hypothesis was OT would amplify both secure and insecure attachment representations in comparison to the placebo condition.

3.5 Ethical consideration

The project study received the approval of the ethics committee from Pirkanmaa Hospital District in December 2014. All participants had been informed and given written consents when participating in the project.

3.6 Statistical methodology

Statistical analysis was performed with SPSS for Windows version 24.0 (IBM, Armonk, NY). The N170 and EPN amplitude data were analyzed with a 2 x 2 x 2 x 2 repeated-measures analysis of variance (ANOVA) with *Face* (infant vs adult), and *Expression* (Happy vs. sad/distressed), *Condition* (OT vs. PL), and *Hemisphere* (left vs right), as within-subjects factors. The LPP data were analyzed with 2 x 2 x 2 ANOVAs with the factors *Condition, Face* and *Expression*. Shapiro-Wilk test was used for checking the normality of distribution of variables. Mauchly's test was included to check the if sphericity assumption was violated. In case of the existence of main effects and interaction, further post hoc *t*-tests for paired samples were calculated and included Bonferroni corrections. Because it was uncertain about OT's effect on brain responses; some of the previous research has shown increased brain responses following OT administration, but some studies have indicated decreased outcomes. Thus, it was advisable to use the significance level at p-value < 0.05 for all two-tailed tests

Regarding data from two questionnaires (i.e. PBQ and ECR-RS), because of quantitative variables, data was analysed by firstly the correlation test to generate an overview of relationships of variables. Subsequently, paired samples t-test was run to compare two means of the score of each individual in two laboratory visits in order to validate the hypotheses.

Using the above-mentioned methods, the data is analysed and presented in detail in the next chapter.

CHAPTER 4: RESULTS

Having defined which methods of statistical analyses used, the results' chapter was broken down into three main parts (1) overview of characteristics of the study participants (2) the OT's effect on the mothers' recognition and attention-related ERP responses to child facial stimuli and (3) the OT's effect on the mothers' perception of bonding to infants and the attachment to the own mothers. Three ERP components (i.e. N170, EPN and LPP) and two questionnaires, (i.e. PBQ and ECR-RS) were presented sequentially; all focus on the meaning and significance of the findings.

4.1 Characteristics of the sample

There were 52 mothers of infants participating in the study and all were right-handed Caucasians. They were mainly living in an urban area and from middle-class families. The mean ages of mothers and babies were 31.92 years (SD=4.98) and 14.51 months (SD=1.18), respectively. More than half of mothers (55.8%) gave birth to their first child, 30.8% to the second, 9.6% to the third and 1.9% to the fourth or more. Nearly 71.2% of mother finished 18 years of education while only 28.8% had 15 or fewer years in their study time. Average education length was 16.33 years. The majority of participants was middle-incomers (from 30 000 to 69 999 euro per year). The female-male ratio of the infants was approximately equal (51.9% for male and 48.1% for female).

4.2 Effects of OT on the recognition of and attention-related ERP responses to the infant.

In this study, the ERPs were accessed via three measures (1) N170 component, (2) the posterior EPN and (3) LPP component. Among 52 participants, only 38 legitimate ones were included in the ERP analysis. The remaining participants were excluded due to excessive artefacts in EEG data (n=3), technical problems (n=3), experimenter errors (n=1), drop-outs (n=2) and the use of oral contraceptive pills (n=5). Exclusion due to the use of oral contraceptive pills was suggested due to published articles indicating the interference of oral contraceptives on the

sensitivity of OT administration (Montoya & Bos, 2017; Scheele, Plota, Stoffel-Wagner, Maier, & Hurlemann, 2016).

4.2.1 N170 component

After excluding excessive artefacts in the EEG, data from 38 valid participants were included. Four outliers were detected but kept for analyses because their values were not extreme. Shapiro-Wilk test was chosen for assessing normality of all factors, due to the small sample size (n<50). Because majority of p-values (except for the N170 values recorded in infant faces-sad-emotion-OT-left hemisphere variable and in adult face-sad emotion-placebo-right hemisphere one) were larger than 0.05, the assumption of normality was not violated. The null hypothesis of Shapiro-Wilk test was that the data's distribution was equal to a normal distribution. In other words, the assumption of normal distribution of the N170-component variable was satisfied for *Face x Emotion x Condition x Hemisphere* within-subject factors. For two variables which were not normally distributed, since factorial repeated ANOVA was fairly robust to violated normality (https://statistics.laerd.com), they were kept intact for analyses. In contrast to expectations, no statistically significant interaction was found for *Face x Emotion x Condition x Hemisphere* within-subject factors in the N170 component, F (1, 37) = 0.001, p-value=0.97. Interestingly, the interaction *Face x Condition* was found to be marginally significant, F (1, 37) =3.30, effect size=0.08, p-value=0.07. Generally, the infant face held more pronounced mean N170 amplitude than the adult ones. Under the OT condition, infant faces elicited more negativity in the N170 waveform than placebo one (-7.14±0.52 µV versus -6.66±0.51µV, respectively)(Table 1).

Additionally, three simple main effects of *Face, Emotion* and *Condition* were found. The simple main effect of *Face* showed a statistically significant difference in infant versus adult faces, which were recorded in the N170 component, F (1, 37) =38.64, effect size=0.51, p-value <0.001. For *Emotion*, its main effect was statistically different between sad/distressed and happy emotions regardless of face's age, F (1, 37) =43.21, effect size=5.4, p-value<0.001. Comparably, the N170 amplitudes for the OT and placebo conditions were significantly

different with F (1, 37) =15.11, effect size=0.16, p-value=0.01. Generally, mean amplitudes of the infant faces, the sad emotion and the OT condition held the larger negativity than their counterparts in N170 measurement (Table 2).

Table 1: The *Face x Condition* interaction recorded in the N170 waveform

Face	Condition	Mean	Std. Error	95% Confidence Interval	
				Lower Bound	Upper Bound
Infant	OT	-7.14	0.52	-8.19	-6.10
	PL	-6.66	0.51	-7.70	-5.63
Adult	OT	-6.16	0.51	-7.19	-5.13
	PL	-6.01	0.49	-7.01	-5.02

Table 2: Three simple main effects of *Face*, *Emotion* and *Condition* under the N170 waveform.

Variables		Mean N170 (μV)	Std. Error	95%CI	p-value
Face	Infant	-6.90	0.50	[-7.93, -5.87]	<0.0001
	Adult	-6.09	0.49	[-7.09,-5.08]	
Emotion	Happy	-6.15	0.49	[-7.15, -5.15]	<0.001
	Sad	-6.84	0.50	[-7.87, -5.81]	
Condition	OT	-6.65	0.50	[-7.67, -5.63]	0.01
	Placebo	-6.34	0.49	[-7.35,-5.33]	

4.2.2. Early Posterior Negativity (EPN) component

Factorial repeated ANOVA revealed that neither the interaction for four within-subject factors *Face x Emotion x Hemisphere x* Condition nor any three-factor interactions in EPN component was found significance, F(1, 37)= 2.42, p-value=0.13. Interestingly, one two-way interaction *Face x Hemisphere*, F(1,37)=4.65, p-value=0.038 was shown to be statistically significant. It is noteworthy that the EPN is interpreted as "relative negativity" which literally means smaller amplitude reflecting the greater attentional response. Therefore, the *post hoc* analysis revealed that the infant faces elicited pronounced EPN compared to the adult faces, which meant that infant faces captured more attention from mothers than adult faces. Moreover, the signals of EPN values recorded in the left hemisphere were showed consistently higher than the ones in the right hemisphere (Table 3).

Table 3: The mean EPN amplitudes in the *Face x Hemisphere* two-way interaction

Face	Hemisphere	Mean EPN (µV)	Std. Error	95% Confidence Interval
Infant	Left	1.66	0.45	]0.75-2.57]
	Right	2.54	0.47	[1.58-3.49]
Adult	Left	2.82	0.45	[1.89-3.74]
	Right	3.99	0.49	[2.98-4.99]

In addition to the *Face x Hemisphere* interaction, there were three simple main effects of *Face, Emotion* and *Hemisphere*. The simple main effect of *Face* ,(F(1, 37)=106.75, effect size=0.74, p-value <0.001), showed that mean EPN amplitudes in infant faces were larger than in adult faces (2.10±0.42µV and 3.40±0.44µV, respectively). For *Emotion*, its main effect was statistically different between the sad/distressed and the happy emotions regardless of face age, p-value=0.006, in which the sad/distressed emotion triggered more pronounced EPN waveforms than the happy emotion (2.65±0.43µV versus 2.85±0.42µV). The EPN elicited by the left hemisphere was significantly augmented compared to the right hemisphere (2.24±0.44 µV and 3.26±0.48 µV, respectively p-value=0.008) (Table 4).

Table 4: Mean EPN amplitudes of simple main effect's factors, separately for *Face*, *Emotion* and *Hemisphere*.

Factors		**Mean EPN(μV)**	**Std Error**	**95% CI**	**p-value**
Face	Infant	2.10	0.42	[1.25-2.95]	<0.001
	Adult	3.40	0.44	[2.51-4.30]	
Emotion	Happy	2.85	0.42	[2.00-3.70]	0.006
	Sad/distress	2.65	0.43	[1.77-3.53]	
Hemisphere	Left	2.24	0.44	[1.34-3.14]	0.008
	Right	3.26	0.48	[2.29-4.24]	

Due to the appearance of two-way *Face x Hemisphere* interaction in four-way repeated ANOVA, we zoomed in the relationship among *Face x Emotion x Condition* separately for the left and right hemispheres. The assumption of normality for all variables was met as assessed by Shapiro-Wilk and no extreme outliers were recorded. However, no statistically significant interaction among *Face x Emotion x Condition* was found for both left hemisphere ($F(1, 37)=1.55$, p-value=0.22) and right hemisphere, and ($F(1,37)=0.002$, p-value=0.96). Two simple main effects of *Face* and *Emotion* were found for the EPN amplitudes recorded in the left hemisphere, in which infant face and sad/distressed emotion revealed higher mean EPN amplitudes (1.66±0.44 vs. 2.82 ±0.45μV, p-value<0.001 and 2.10±0.45 μV, p-value=0.001, respectively). In the right hemisphere, only one main effect of *Face* was recorded, $F(1, 37)=102.93$, p-value< 0.001, in which infant faces had higher mean EPN amplitudes than adult faces (2.54±0.47 μV and 3.99±0.49 μV, respectively) (table 5).

Table 5: Main effects of the EPN analysis for the left and right hemispheres, separately

Left hemisphere			**Mean**	**Std. Error**	**95% Confidence Interval**	**p-value**
	Face	Infant	1.66	0.44	[0.75-2.56]	<0.001

		Adult	2.82	0.45	[1.89-3.73]	
	Emotion	Happy	2.38	0.44	[1.48-3.27]	0.001
		Sad/distressed	2.10	0.45	[1.18-3.01]	
	Condition	OT	2.19	0.46	[1.12-3.13]	0.73
		Placebo	2.28	0.46	[1.33-3.23]	
Right hemisphere	Face	Infant	2.54	0.47	[1.58-3.49]	<0.001
		Adult	3.99	0.49	[2.98-4.99]	
	Emotion	Happy	3.32	0.47	[2.34-4.27]	0.15
		Sad/distressed	3.20	0.49	[2.20-4.20]	
	Condition	OT	3.10	0.49	[2.10-4.11]	0.23

4.2.2 Late Positive Potentials (LPP) component

The main finding with regard to the LPP component was no statistically significant interaction found among three stimuli *Face (Adult/Infant) x Emotion(Happy/Sad) x Condition(OT/Placebo)*, F(1, 37)= 0.46, effect size =0.12 p-value=0.5. It indicated that neither face stimuli nor emotion stimuli showed any differences in the LPP strength in either OT or placebo. OT had no effects on enhancing the facial or emotional perception in the mothers. However, the simple main effect of *Emotion* showed a statistically significant mean difference in the sad/distressed (3.08±0.31 µV) versus the happy emotions (2.82±0.29µV) which were recorded in LPP components, F(1, 37)=8.86, p-value =0.005. Similarly, the simple main effect of *Face* indicated that mean LPP strength displayed statistically different between infant faces and adult faces, (3.11±0.31µV vs. 2.80±0.30 µV, respectively), F(1, 37) =8.83, p-value=0.005.

Overall, all of three ERP components demonstrated none significant interactions relevant to OT's effect. Only in the N170 waveform, the appearances of a marginal interaction *Face x Condition* and the significant main effect of *Condition* is noticeable because they are related to the research question.

4.3 Questionnaire data: effect of OT on perception of bonding/attachment of the mothers to the infant.

The analyses of participants' questionnaires were separated from the analysis of the ERP data (i.e. one value excluded in the ERP analysis might still be included in the questionnaire data as long as it contained the valid values). Exclusion criteria in the ERPs such as oral contraception uses, artefacts or technical problems did not apply to questionnaire assessments. Participants completed two questionnaires at the end of laboratory visit. Table 6 summarized the results of questionnaire data.

Table 6: The relationship of PBQ score and two elements of ECR-RS score in both conditions.

	N	Mean	Std. Deviation	p-value
PBQ score in OT	49	49.69	3.57	0.60
PBQ score in placebo	52	49.42	3.1	
Avoidance score of ERC-RS in OT	47	2.55	1.34	0.82
Avoidance score of ERC-RS in placebo	47	2.63	1.46	
Anxiety score of ECR-RS in OT	50	1.43	0.97	0.34
Anxiety score of ECR-RS in placebo	50	1.37	0.84	

4.3.1 Impaired mother-infant bonding on Postpartum Bonding Questionnaire.

The PBQ is a self-rating questionnaire which is designed to detect an early mother-infant relationship disorder. It contains 25 statements with the six-point score ranging from zero ("Always") to five ("Never") on four sections: (1) a general factor/impaired bonding (twelve items), (2) rejection and anger (seven items),(3) anxiety about care (four items) and (4) risk of abuse (two items) (Brockington et al., 2006).

In this study, we utilized only the *impaired bonding* section of the PBQ, i.e. a general factor, which contains only an 11-item questionnaire, to access mother-infant bonding (see Appendix 1). In this study, the term of "PBQ score "or "PBQ test" indicated only the subscale of 11-

items *impaired bonding* section. For positive statement, the higher score the statement had, the close the mother-infant bonding was. In contrast, the score was reversed in negative statements, i.e. the more negative statement, the higher score, which reflected distressed emotions or attitudes. Therefore, the higher score indicated the problematic mother-infant bonding. In this subscale, 55 was the highest possible score which participants could have while zero was the lowest one. We also used the threshold of 12 as the cut-off score for detection of impaired bonding as suggested by (Brockington, et al., 2001). All participants (n=52) filled in two PBQ forms, one in placebo and the other in OT condition.

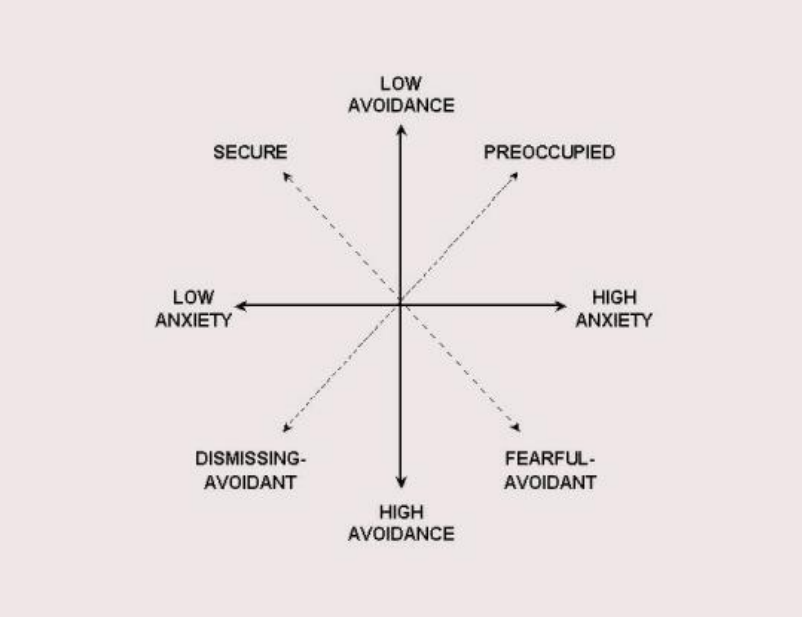
Specifically, all variables were quantitative, thus correlation was the preliminary statistical test to measure associations between two variables. A quick run of Spearman correlation, which was used for skewed distributions, between two PBQ scores manifested that a strong positive correlation ($r=0.8$, $p<0.001$, $n=49$) between two PBQ scores in both OT and placebo conditions was recorded. This indicated that high score of PBQ in placebo would predict the high score in the OT condition. A paired-sample t-test was then used to analyze if there were statistically significant mean differences in the PBQ score between the OT condition and placebo. There were 49 valid values and two outliers were detected as assessing via plot box; however, their values were not considered extreme, thus they were kept in the analysis. The assumption of normality was not violated as assessed by Shapiro-Wilk's test ($p=0.11$). Unfortunately, the result suggested that there was no statistically significant difference in the *impaired bonding* of PBQ score between the mothers who received intranasal OT and those with placebo ($p=0.60$). Therefore, the hypothesis that OT may increase perception bonding in mothers was not supported by the PBQ data.

4.3.2 Experiences in Close Relationship-Relationship Structure.

The ECR-RS is a self-rating questionnaire which assesses two-dimensional attachment pattern: avoidance and anxiety, which mutually form the secure and insecure attachment. (Figure 2) This instrument serves as a robust measure of adult attachment, assessing attachment-related avoidance and anxiety dimension in four close relationships (e.g. mother, father, romantic

partner and best friend). Each distinct section consists of 9-item statements corresponding to the single type of close relationship (Fraley et al., 2011). In this study, the participants were asked only about their close relationship to mothers.

Figure 2. Two dimension of anxiety and avoidance in relation to secure-insecure relationships.



(credit by R. Chris Fraley on website http://internal.psychology.illinois.edu/~rcfraley/measures/ecrr.htm)

Firstly, due to the skewed distribution, a Spearman correlation was run to investigate the correlation between two *Avoidance* scores in both OT and placebo conditions. Preliminary analysis showed that the relationship was monotonic as shown visually on the scatterplot (Appendix 3). A strong positive correlation between two *Avoidance* scores in both OT and placebo was found to be statistically significant ($r = 0.86$, $p<0.001$, $n=47$). Similarly, the Pearson correction test for normal distribution was run for *Anxiety* scores under both conditions. Interestingly, the correlation was displayed to be positively weak between two variables (i.e. two *Anxiety* scores in OT and in placebo) ($r=0.69$, $p<0.001$, $n=47$). Therefore, it is unlikely that the OT has profound effects on either *Anxiety* or *Avoidance* scale of ECR-RS

score in mother relationship. Regardless of conditions, the score of ECR-RS in placebo was positively correlated to the score in OT condition.

Secondly, the paired samples t-test was conducted to compare mean scores of *Avoidance*-relate attachment in both OT and placebo conditions. There were 47 valid variables in the analysis one outlier but the outlier was kept in the analysis. However, there was no statistically significant difference between two means of *Avoidance* scores in both conditions ($p = 0.82$). Similarly, the means of *Anxiety* scores recorded under the OT nasal admiration and placebo were found insignificantly different ($p = 0.34$). Combined two results, it was likely that OT affected neither *Avoidance*-related nor *Anxiety*-related attachment in ECR-RS score. In other words, the hypothesis of OT amplifying secure/insecure attachments was unsupported empirically in the present analysis.

In short, it is somewhat surprising that both the PBQ and ECR-RS questionnaire data did not find any significant differences in the scores obtained in OT and placebo condition.

Taken together, the results in this chapter indicate that the clear effects of OT in both enhancing neural processing in the mothers toward infant stimuli (via the ERP data), and promoting subjective bonding/attachment's perceptions of the mothers (via questionnaires) could not be statistically identified in this analysis. The next chapter, therefore, moves to evaluate the results as looking back initials hypotheses and then comparing the findings with different studies in the literature.

CHAPTER 5: DISCUSSION

This chapter compares the results obtained from the previous chapter to the initial hypotheses to see if the experimental evidence supports the proposed hypotheses. Furthermore, the key findings are then compared and contrasted to preceding studies in the literature. Subsequently, the strengths and the weakness of the thesis study in terms of study design, and its contribution to the current evidence of OT are mentioned as well. Finally, the need for further studies and successive research is suggested briefly to overcome the current study's limitations.

5.1 Comparison between my study and initial research questions.

The thesis study has two research questions corresponding to two data analyses: the ERPs and questionnaires. The ERP analysis was performed to test a hypothesis that intranasal OT promotes the recognition and attention-related ERP responses of the young mothers toward infants' emotional stimuli. The reason behind this task is an adaption extended from one published study by Peltola et al. (2014), which demonstrated that both motherhood and the genetic variation of the OT receptor gene were specifically associated with promoted ERP responses to only emotionally elective infant expression (i.e. facial expressions particularly in sad/distressed faces), but not adult faces.

Firstly, the first research question corresponded to the analysis of three ERP components, N170, posterior EPN and LPP, which revealed no significant interaction among stimuli (i.e. *Face x Emotion x Hemisphere x Condition* for N170 and EPN and *Face x Emotion x Condition* for LPP component). The *Face x Condition* interaction was marginally statistically significant in the N170 component (p-value=0.07). This marginal significance implied that there was a difference in mean amplitudes of N170 recorded in the OT and placebo condition, in which the amplitude of N170 increased (e.g. more negative amplitude) under the OT's presence. However, the experimental support for the OT's effect is fairly vague in the present analysis. Otherwise, if the OT's effect does exist, it may be very minute. Besides, there was one interaction between *Face* (infant vs. adults) and *Hemisphere* (left vs. right), which showed to

be significant in the posterior EPN. Nonetheless, this interaction is out of interest because the initial research question focuses on the OT's effect.

Specifically, the OT condition showed only the main effect recorded in mean N170 amplitude, in which OT held larger negativity than placebo. No other main effects or significant interactions involved in OT appeared in other ERP components. In the analysis, the infant faces displaying the sad/distressed emotion captured the higher attention of the mothers than the adult ones. However, this effect was independent of the OT's presence. Hence the study findings do not support the hypothesis of the effect of OT on enhanced recognition and attention of the mothers toward infant's emotional negative signals.

Secondly, with regard to two questionnaire data (i.e. PBQ and ECR-RS), the research question was raised to test if OT influenced on the mothers' perceptive relationship with their babies. However, the PBQ scores recorded in the mothers receiving OT and those received placeboes showed no statistical difference. This means OT does not give any impacts on mothers' perception of bonding relationship to their infants. When participants recalled their relationship with their own mothers and filled the ECR-RS questionnaire, the results also yielded no significance on both *Avoidance* and *Anxiety* scores (i.e. two elements of ECR-RS) regardless of substances received. Therefore, it is unlikely that the outcome of both questionnaires support the hypothesis about the effects of OT on perceptions and bonding.

Conclusively, results from both ERP analysis and the questionnaire data are consistently demonstrated that none of the results reaches significant levels in order to empirically support the initial research questions about the OT's effects.

5. 2 Comparison with other studies.

5.2.1 The ERPs.

The ERP task is designed according to the study by Peltola and colleagues (2014), which had the similar experimental settings with adult and infant photographic stimuli. The difference is

the participants received intranasal OT/placebo before entering the ERP task in this study. Regarding photographic facial stimuli, the ERP results are consistent with the one in Peltola's study, in which the posterior EPN amplitude was significantly augmented in infant faces displaying the negative emotion, compared to adult ones (Peltola et al., 2014). Statistically speaking, LPP data shares similarities with outcomes of a recent study by Rutherford *et al.* (2017) using a similar experimental design; in that study, LPP waveforms appeared to be unaffected by OT, relatively compared to placebo (Rutherford et al., 2017). Interestingly, no interaction between OT and other variables was reported in Rutherford's research whilst borderline significant interaction between *Face* and *Condition* is found in this study. It is noteworthy to mention that participants in Rutherford's study were non-mother healthy people while the ones in this present study were young healthy mothers. Thus, the effect of OT on the infant vs. adult face perception may be present solely in mothering people although it can be encoded at very some modest levels.

Additionally, no significant difference between the healthy mothers administered intranasal OT toward infant faces showing negative emotions and those administered placeboes was found in the thesis study. This finding is in line with the conclusion of a recent meta-analysis of seventeen studies, pointing out that OT has no significant effect on emotion recognition (Keech, Crowe, & Hocking, 2018). However, all participants are diagnosed with clinically social disorders, not being healthy population like this study.

However, the findings of insignificant effects of OT are contradictive to established literature, which usually hypothesizes the OT does sensitize adults to infant cues. One study by Marsh et al. (2012) used similar experimental settings with a double-blind design and infant/adult-face photographic stimuli (Marsh et al., 2012). It indicated that the adults received intranasal OT found infant faces more appealing than the ones received placebo, whereas infant faces were rated more preferred. Another study with a double-blind, within-subject experiment revealed intranasal OT administration enhanced responsive interactions of fathers with babies in a more caring manner compared to placebo ones (Naber, van IJzendoorn, Deschamps, van Engeland, & Bakermans-Kranenburg, 2010). Nonetheless, those studies used different experimental

measurements (task-performance for the first-mentioned and questionnaire for the second one, respectively), thus it can possibly explain the inconsistency.

Besides, neurological research with the use of neural-measurement techniques for OT inhalation displayed contradictive findings to ours. One randomized controlled trial study by Riem et al. (2011) ,using fMRI technique and OT nasal spray, investigated the effect of OT on neural responses to infant crying. Results indicated that induced OT reduced the activation of the amygdala and increased the activation of the insula and the inferior frontal gyrus (Riem et al., 2011). Interestingly, in the same experimental context when the infant crying was labelled differently ("sick" vs. "bored"), OT showed opposite results in different parts of the brain activity. Indeed, in case of "sick"- labelled infants' crying, OT promoted empathetic reactions while reducing perceived urgency toward crying of "bored"-labelled infants (Riem et al., 2014). Yet, compared to this thesis study, the different method used to assess recognition, i.e. study design (between-subject and within-subject, respectively), neural experimental techniques (fMRI vs. EEG, respectively) and stimuli (sound vs. picture, respectively), may be a possible explanation for the diverging effects of OT on brain activities under the nasal route.

Additionally, there may be the genetic explanation behind the silent effect of the intranasal OT compared to placebo in mothers' ERP responses. Numerous studies revealed experimental evidence indicating that genetic variants of the OT-pathway genes do influence optimal parental behaviours. In a study with laboratory, solving-tasks showed that mothers, who were the GG allele carriers of OT receptor gene (called *OXTR*), engaged in more sensitive interactions with their children than the other allele-carrying mothers (Mileva-Seitz et al., 2013). Likewise, only those having GG homozygotes on *OXTR* allele preferred infant faces to adult faces after OT inhalations (Marsh et al., 2012). In addition, the study of OT inhalation by Marsh *et al.* (2012) emphasized that effect of OT on the preference of infant's face to adult' face was only observed on GG allele carriers (Marsh et al., 2012). Finally, in ERP signals recorded from mothers vs. non-mothers responding to infant vs. adult faces, Peltola *et al.* (2014) indicated that only the GG allele of the *OXTR* genotype showed an early-latency differential frontal response to negative infant facial expressions, but not to adult faces. This

suggested that different variants of the *OXTR* gene might modulate differential brain responses to adults' and infants' emotional cues. Sadly, in the thesis study, no genotype analysis tests were performed to examine the genetic genotypes of the participants, thus it is unlikely to estimate the prevalence of GG allele in the study population.

Importantly, even in case of GG-allele carriers, the poor phenotype of *OXTR* may be influenced by environmental impacts, i.e. epigenetics. Majority of genetic effects were modulated by gene-environment interaction rather than by the main effects themselves. Thus, it can be explainable for no-response to OT in mothers. Therefore, even the recent meta-analysis could not draw conclusive findings of the significance of *OXTR* allele due to failures to investigate epigenetic modulations of the OT-pathways gene (Feldman, Monakhov, Pratt, & Ebstein, 2016). Furthermore, the emergence of diverging-and-even-contradictory results has been a big problem in OT research, reported by many meta-analyses (Keech et al., 2018; Leppanen, Ng, Tchanturia, & Treasure, 2017; Valstad et al., 2017). Effect of OT on social-emotional function should be thus deployed in contextualized and individual differences (Bartz, Zaki, Bolger, & Ochsner, 2011). Criticism is directed at lack of a theoretical framework to detect contradictory findings and poor understandings about OT pathway to the brain (Leng & Ludwig, 2016).

5.2.2 The questionnaire data.

Performance of two questionnaires (PBQ and ECR-RS) is supplementary tasks which were preliminarily designed to support the proposed hypothesis of enhanced effects of OT to bonding perception of young mothers. Those questionnaires were chosen to be complementary to each other. Investigating secure/insecure attachment styles of participants when thinking about mothers (i.e. via ECR-RS questionnaire) may give hints to explore infant-mother bonding perception of participants (i.e. via PBQ questionnaire). The results, however, are unsupportive to the hypothesis; they are consistently in agreement with the findings of the ERPs, which are found no significant difference in performances between OT- and placebo-receivers.

Firstly, the PBQ was initially developed by Brockington et al. (2001) and gradually used as a screening tool in predicting symptoms of a" disorder of the mother-infant relationship" (Brockington et al., 2001). The questionnaire consisted of 25 items which were grouped into four sections of clinical relevance used to construct four scales and each scale was set up specific threshold. Brockington *et al.*(2006) emphasized that bonding disorder was among various type of emotional disorders appearing in mothers during the postpartum period. Though bonding disorder and depression were usually reported to be correlated, it was not always a comorbidity of depression (Brockington et al., 2006; Moehler, Brunner, Wiebel, Reck, & Resch, 2006). Then, subsequent studies have adapted PBQ into different language versions based on societies and cultures such as Japan (Suetsugu, Honjo, Ikeda, & Kamibeppu, 2015) and Italy (Busonera, Cataudella, Lampis, Tommasi, & Zavattini, 2017) but the validity and reliability have been still confirmed. In this thesis study, our goal is not to investigate the validity and reliability of the PBQ on Finnish population, but we test if OT has any effect on mothers' perception of bonding to her child, thus only the general factor is used. This section is concerned with *impaired bonding*, which serves to identify some kinds of problem of mother-infant relationship. Unfortunately, neither mothers received OT nor ones received placebo showed the significant difference in PBQ score.

Secondly, the ECR-RS was extracted to focus on the mothering relationship of the participants to their mothers via exploring secure/insecure attachment relationships. The questionnaire data showed no effect of OT found in *Avoidance* and *Anxiety* scores (which represented secure/insecure attachments) compared to placebo. Again, the result disagrees with one study having 26 healthy male students, who viewed Adult Attachment Projective Picture System (the AAP) then ranked. A single dose of OT administration was found to significantly increase the experience of the secure attachment on the students (Buchheim et al., 2009). However, a number of studies have indicated that positive effect of OT can be minute or less pronounced in individuals experienced (relatively) negative childhood such as harsh parenting or high love-withdrawal (Bakermans-Kranenburg, van IJzendoorn, Riem, Tops, & Alink, 2011). Moreover, inter-individual differences, such as individual characteristics and experiences, can fluctuate effects of OT (Bartz et al., 2011; Olff et al., 2013). Therefore, it suggests personal traits,

attachment styles, genders and psychopathology should be taken into account as considering OT's influence on promoting pro-social behaviours (Shamay-Tsoory & Abu-Akel, 2016); therefore, the contradiction in results of questionnaire assessment on social behaviours can be partially understandable.

In sum, comparing to preceding studies in the literature, it turns out to be blended results for both the ERPs' and questionnaires' results with regard to the OT's influence on emotional recognition and perception of parents toward infant cues, some of which agreed with the current analysis and the other disagreed. Moreover, genetic information and epigenetic signature can contribute to the mixed effects of OT.

5. 3 Strengths and limitations of the thesis study

A major strength of the thesis study is its well-controlled, double-blind and within-subject design. Allocation of substances (i.e. OT or placebo) is blindly distributed to both participants and staff members, thus the bias of self-awareness is minimized. Within-subject design allows us to achieve the targeted effect size (d=0.50) while requiring a fewer number of participants to detect the mean difference between two conditions (i.e. OT or placebo). Therefore, it helps to shorten the recruitment period and financially save. In addition, since a high OT's level has been known to be associated with lactation breastfeeding (White-Traut et al., 2009) and it has been considered a confounder in many OT research, we rule out this breastfeeding-related possibility when including participants into the study. Because there is one study suggested that hormonal contraceptive pills might interfere effects of OT (Scheele et al., 2016), thus the data obtained from participants using the pills are excluded in the present ERP analysis. The possible confounder due to the use of contraceptive pills is prevented from the beginning.

However, the study has also some limitations which should be taken into account when evaluating its value. Firstly, it is performed in the randomized control trial (RCT) pattern, thus drawback of RCT is also associated with this study. RCTs have typically included homogenous samples of individuals via strict inclusion and exclusion criteria, thus the

generalizability to broader populations i impeded (Keech et al., 2018). Secondly, our study population biases on the population of women who either visit the Tampere University Hospital (TAYS) or live in the Pirkanmaa region. As stated previously, the lack of information about the genetic pattern of the OT-related genes may be the biggest loophole of this study because some literature evidence suggests individual's variations in OT may contribute to OT responses. Last but not least, regarding data from the self-reported questionnaires, reporting bias is unlikely to estimate and prevent; problems associated with self-report methods including subjectivity and social desirability bias are also inevitable.

Replication of the experimental design with a larger population in one future study may be good tactics to increase the reliability of the effect of OT found on the N170 waveform if it does exist. Then, more studies on either clinical or non-clinical population are required to build up concrete evidence for OT's clinical significance in treatments of parenting difficulties.

In summary, this chapter is divided into three main sections, each of which reflects the author's viewing angle and contributes to the overall evaluation of the thesis study's results. The next chapter summarizes and brings together all description, findings and discussion depicted in the main areas of the thesis. Furthermore, it gives final comments, and suggestions for improvement of future work's direction.

CHAPTER 6: CONCLUSIONS

The thesis study is a subset of the project study, primarily aimed at investigating the effect of intranasal OT (versus placebo) administration on mothers' neural and behavioural responses to child's emotional cues via the ERP technique. Analysis of the ERP data yielded no significant difference between OT and placebo conditions, except one marginal significance of *Face x Condition* interaction found in the N170 component. In addition, the main effect of *Condition* appeared significantly only in the N170 component, which indicated the OT held larger negativity than placebo. Therefore, the hypothesis of OT enhancing mothers' neural responses is faintly supported by the present analysis. Furthermore, the secondary aim was to test whether the OT influence subjective perceptions of bonding in the relationship between mother-infant and mother-to-owned-mother, by employing the PBQ and ECR-RS questionnaires. Similar to the ERP findings, no significant difference in terms of questionnaires' scores was found between OT and placebo. The strength of the study results from the well-controlled design, which minimizes several possible confounders. However, its weakness is from lack of genetic information of participants, thus hardly understanding the gap between observed OT's effects and their underlying genetic background.

Generally speaking in terms of the value, the thesis study has been one of the very few studies using the ERP technique to examine the OT's effects on women's neural processing of facial stimuli. Thus, its results contribute firstly to the project study which includes other non-ERPs tasks, then secondly to the currently mixed literature of the OT's influence on pro-social and parental behaviours.

More broadly, future studies could be conducted in different populations (e.g. non-Finnish, non- Caucasian mothers or multiparous mothers). In this study, the majority of mothers are primiparous, e.g. gave birth to their first child. A successive study with a larger study population can help to increase the reliability of the ERP results. In addition, it is interesting and necessary to include genetic information and the epigenetic signature in future works in

order to capture dynamic effects of OT in reality. The full scale of the PBQ and ECR-RS questionnaires can be used in other studies to verify their validity in the Finnish population

CHAPTER 7: APPENDICES

7.1 Subscale of Post-Partum Questionnaire

Subscale of Impaired bonding in Postpartum Bonding Structure (adapted from (Brockington, Fraser, & Wilson, 2006).

The Finnish translated version was provided to all participants. This questionnaire reflected the content of statements. The 10th statement was excluded from the original form.

Please indicate how often the following are true for you. There is no "right" or "wrong" answers. Choose the answer which seems right in your recent experience

Scoring	Statement	Always (0)	Very Often (1)	Quite Often (2)	Sometimes (3)	Rarely (4)	Never (5)
0->5	I feel close to my baby						
5->0	I wish the old days when I had no baby would come back						
5->0	The baby does not seem to be mine						
5->0	My baby wind me up						
0->5	I love my baby to bits						
0->5	I feel happy when my baby smile or laugh						
5->0	My baby irritated me						

5->0	My baby cries too much
5->0	I feel trapped as a mother
~~5->0~~	~~I resent my baby~~
0->5	My baby is the most beautiful baby in the world
5->0	I wish my baby would somehow go away.

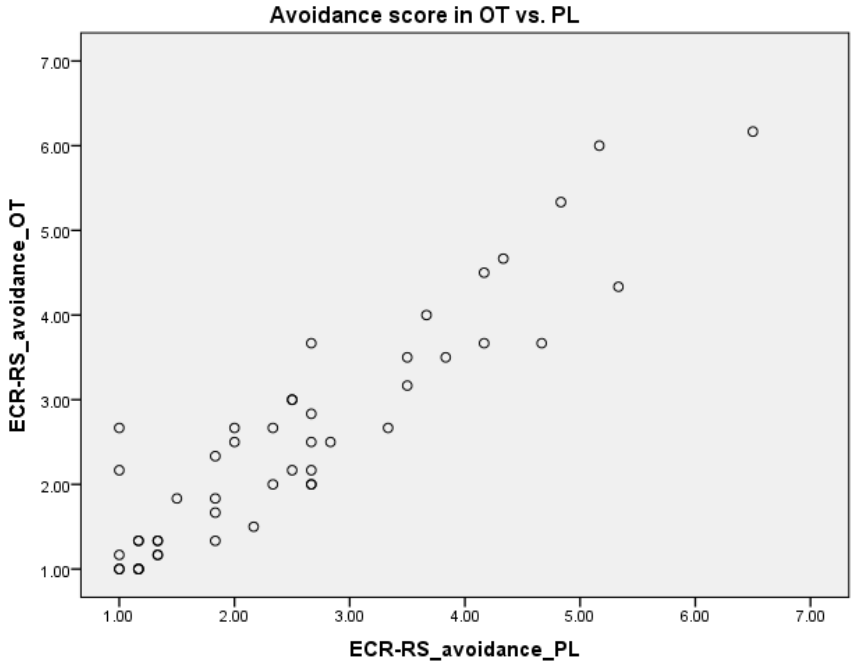
7.2 Experiences of Close Relationship-Relationship Structure

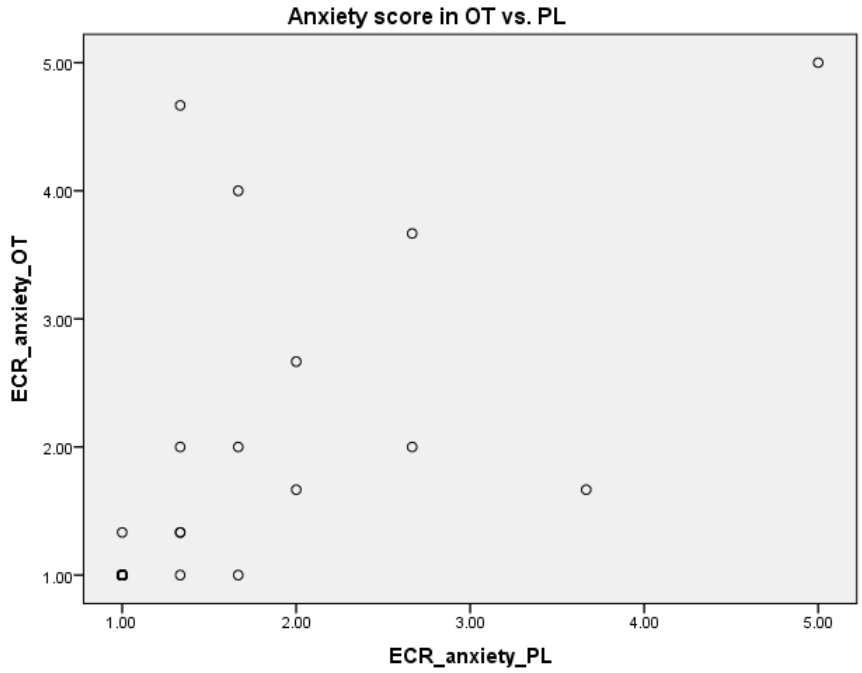
Experiences of Close Relationship-Relationship structure (adapted from Relationship Structures (ECR-RS) Questionnaire by R. Chris Fraley (http://internal.psychology.illinois.edu/~rcfraley/measures/relstructures.htm, retrieved 08/11/2017)

The original ECR-RS has one section for "general" attachment then four sections of relationship-specific attachment (i.e. mother, father romantic partner and best friend). Herein, the study used only the section of mother-like relationship and participants were asked response the questionnaire when thinking of their own mothers. The instruction used to assess those section was "Please read each of the following statements and rate the extent to which you believe each statement best describes your feelings about **your mother".** The first 6 items are designed for *Avoidance* score with the first 4 items reverse keyed (because the last two items are negative statement) whilst the last 3 items record *Anxiety*. The answers falls into five categories: (1) strongly disagreed,(2) disagree, (3) neither agree nor disagree, (4) agree and (5) strongly agree.

Scoring/ Statement	strongly disagreed (1)	Disagree (2)	Neither agree nor disagree (3)	Agree (4)	strongly agree. (5)
1. It helps to turn to people in times of need.					
2. I usually discuss my problems and concerns with others.					
3. I talk things over with people.					
4. I find it easy to depend on others.					
5. I don't feel comfortable opening up to others.					
6. I prefer not to show others how I feel deep down.					
7. I often worry that other people do not really care for me.					
8. I'm afraid that other people may abandon me.					
9. I worry that others won't care about me as much as I care about them					

7.3 Scatterplot of *Avoidance/Anxiety* scores in both OT and placebo conditions





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